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PERTANIKA JOURNAL OF TROPICAL AGRICULTURAL SCIENCE

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Overview

Pertanika Journal of Tropical Agricultural Science is an official journal of Universiti Putra Malaysia. It is an open-access online scientific journal. It publishes the scientific outputs. It neither accepts nor commissions third party content.

Recognised internationally as the leading peer-reviewed interdisciplinary journal devoted to the publication of original papers, it serves as a forum for practical approaches in improving quality on issues pertaining to tropical agriculture and its related fields.

Pertanika Journal of Tropical Agricultural Science currently publishes 6 issues per year (*January, February*, *May*, *June, August*, and *November*). It is considered for publication of original articles as per its scope. The journal publishes in **English** and it is open for submission by authors from all over the world.

The journal is available world-wide.

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2. The Chief Executive Editor sends the article-identifying information having been removed, to 2 or 3 reviewers. They are specialists in the subject matter of the article. The Chief Executive Editor requests that they complete the review within 3 weeks.

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3. The Editor-in-Chief examines the review reports and decides whether to accept or reject the manuscript, invite the authors to revise and resubmit the manuscript, or seek additional review reports. In rare instances, the manuscript is accepted with almost no revision. Almost without exception, reviewers' comments (to the authors) are forwarded to the authors. If a revision is indicated, the editor provides guidelines to the authors to attend to the reviewers' suggestions and perhaps additional advice on how to revise the manuscript.

4. The authors decide whether and how to address the reviewers' comments and criticisms and the editor's concerns. The authors return a revised version of the paper to the Chief Executive Editor along with specific information describing how they have answered' the concerns of the reviewers and editor, usually in a tabular form. The authors may also submit a rebuttal if there is a need especially when the authors disagree with certain comments provided by the reviewers.

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Foreword

Welcome to the fourth issue of 2026 of the Pertanika Journal of Tropical Agricultural Science (PJTAS)!

PJTAS is an open-access journal for studies in Tropical Agricultural Science published by Universiti Putra Malaysia Press. It is independently owned and managed by the university for the benefit of the world-wide science community.

This issue contains 25 articles: one short communication; one review article; and the rest are regular articles. The authors of these articles come from different countries namely Africa, Brunei Darussalam, China, Indonesia, Malaysia, Nigeria, Pakistan, Philippines, Thailand, and Vietnam.

The regular article entitled "Evaluating Ovarian Maturation Stages of Banana Shrimp, *Penaeus merguiensis*" presents a comprehensive morphological and histological characterisation of female banana shrimp (*P. merguiensis*) to support broodstock selection and captive breeding programs. Through visual observation and tissue analysis across 50 female specimens, the study established five distinct ovarian developmental stages: immature, premature, mature, fully mature, and spent. Morphologically, ovaries progressed from transparent to yellowish-white, light green, dark green, and finally pale cream, which correlated with significant increases in both the gonadosomatic index (GSI) and oocyte diameter up to the fully mature stage. Histologically, the transition was marked by the shift from perinucleolar and yolkless oocytes to yolky oocytes, with fully mature ovaries uniquely featuring elongated cortical rods essential for egg protection during fertilisation. These findings clarify the vitellogenesis process in *P. merguiensis*, offering valuable baseline metrics for optimising timing in wild broodstock harvesting, spawning induction, and sustainable aquaculture management. Further details of this study can be found on page 1567.

The regular article entitled "Life Cycle Assessment of Conventional Broiler Farm System in Malaysia and Carbon Footprint Mitigating Strategies" evaluates the environmental impacts of commercial broiler production using a cradle-to-farm-gate Life Cycle Assessment (LCA) framework aligned with IPCC guidelines. Analysing data from an intensive closed-house production system in Johor, Malaysia, the study established a baseline emission intensity of 1.54 kg CO_2-eq per kg of live weight, totalling 63.97 tCO_2-eq per house per 62-day cycle. Hotspot analysis revealed that upstream feed procurement contributed the largest share of

greenhouse gas emissions (39%), followed by operational energy consumption (37%) and on-farm manure management (24%). The authors further modelled three poultry litter (PL) management strategies, finding that while selling raw PL yielded the highest nominal farm-gate carbon reduction (29.87%), converting PL into biochar (20.13% reduction) or compost (15.58% reduction) provided superior long-term environmental sustainability by stabilising organic carbon, preventing nutrient leaching, and suppressing gas volatilisation. Full details of this study are available on page 1577.

The regular article entitled "Assessing the Impact of Land Use on Arbuscular Mycorrhizal Root Colonisation Rate, Phosphorus and Nitrogen Uptake in Ife Bimpe Cowpea" examines how pre-existing soil land-use histories and organic inputs influence arbuscular mycorrhizal fungi (AMF) symbiosis and growth performance in tropical legumes. Utilising soils from three land-use origins—African Star Apple (ASAR), Cassava Root (CRR), and Bush Fallow (BFR)—amended with poultry manure, cow dung, or their combination, the authors evaluated AMF spore abundance, root colonisation rates, and nutrient availability. Results indicated distinct soil- and amendment-specific responses: CRR soils and poultry manure applications significantly enhanced AMF root colonisation (reaching up to 48% and 44%, respectively), whereas ASAR soils and cow dung amendments optimised total spore abundance and *Gigaspora* spp. sporulation. Ultimately, ASAR soils amended with organic manure supported superior vegetative growth by Week 9, highlighting that matching specific organic amendments with land-use histories can optimise AMF-mediated nutrient uptake and crop productivity in nutrient-limited tropical soils. Further detailed information can be found on page 2031.

We anticipate that you will find the evidence presented in this issue to be intriguing, thought-provoking and useful in reaching new milestones in your own research. Please recommend the journal to your colleagues and students to make this endeavour meaningful.

All the papers published in this edition underwent Pertanika's stringent peer-review process involving a minimum of two reviewers comprising internal as well as external referees. This was to ensure that the quality of the papers justified the high ranking of the journal, which is renowned as a heavily-cited journal not only by authors and researchers in Malaysia but by those in other countries around the world as well.

We would also like to express our gratitude to all the contributors, namely the authors, reviewers and Editorial Board Members of PJTAS, who have made this issue possible.

PJTAS is currently accepting manuscripts for upcoming issues based on original qualitative or quantitative research that opens new areas of inquiry and investigation.

Editor-in-Chief
Md Kamal Uddin

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Short Communication

Evaluating Ovarian Maturation Stages of Banana Shrimp, *Penaeus merguiensis*

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ABSTRACT

The present study investigated the ovarian development of female banana shrimp, *Penaeus merguiensis* (n = 50), with a focus on external morphological and histological assessments. The findings indicated that *P. merguiensis* undergoes five distinct ovarian developmental stages: immature, premature, mature, fully mature, and spent. Externally, the ovaries changed in colour from transparent to whitish-yellow, light green, dark green, and finally white again from the immature to spent stages. Histologically, the presence of perinucleolar and yolkless oocytes characterised the immature and premature stages, whereas yolky oocytes predominated during the mature and fully mature stages. The gonadosomatic index (GSI) and oocyte size increased significantly from the immature to fully mature stages before decreasing significantly at the spent stage ($p < 0.05$). Understanding these ovarian developmental stages may contribute to the domestication and reproductive management of this species, particularly for broodstock maturation and seed production.

Keywords: Banana shrimp, ovarian maturation

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INTRODUCTION

Penaeus merguiensis is reported to be one of the most economically important shrimp species and a key catch in Malaysia (Hurzaid et al., 2023). Commonly known as the banana shrimp, *P. merguiensis* is considered a promising candidate for aquaculture in Malaysia due to its firm

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texture and fine flavour. This species is abundantly found in coastal areas and local markets across Malaysia, particularly in the northern regions of Peninsular Malaysia (Kedah, Perak, and Pulau Pinang), the southern region (Selangor and Johor), the eastern coast (Kelantan and Terengganu), as well as in East Malaysia (Sarawak), where it is primarily harvested from the wild by local fishermen (Abdul Halim et al., 2023; Aziz et al., 2020). *P. merguiensis* offers several advantages for shrimp farmers, including its large size (Robalino et al., 2016), rapid growth rate (Vance & Rothlisberg, 2020), and resistance to diseases (Wiradana et al., 2020). Hence, *P. merguiensis* appears to be a suitable candidate for aquaculture due to its wide availability of broodstock in Malaysia and its favourable biological traits. The culture of native species with abundant broodstock resources may support small-scale farmers in rural areas in establishing sustainable shrimp farming operations.

However, there is limited information on the basic reproductive biology of female *P. merguiensis*, which is critical for their use as broodstock in aquaculture. Although reproductive studies have been conducted in several penaeid shrimp species, information on ovarian maturation stages in female *P. merguiensis*, including external morphology, oocyte development, histological characteristics, and gonadosomatic index (GSI), remains limited. Typically, most penaeid shrimp undergo five distinct ovarian maturation stages, which can be detected through visual observation and confirmed more accurately through histological assessment (Manan et al., 2022). Previously, several penaeid species, including the Indian white prawn, *Penaeus indicus* (Amanat & Qureshi, 2011), giant freshwater prawn, *Macrobrachium rosenbergii* (Revathi et al., 2012), and whiteleg shrimp, *Litopenaeus vannamei*, have been reported to exhibit five ovarian maturation stages characterised by distinct ovarian colour changes from translucent, yellow, light green and ending with dark green, accompanied by progressive increases in gonadosomatic index (GSI) and oocyte size with advancing maturation stages. Therefore, the present study aims to provide detailed information on the ovarian maturation of *P. merguiensis* through assessments of external morphology and histology, covering ovarian colour, GSI, oocyte diameter and yolk progression. Identification of ovarian developmental stages can help determine the spawning season and reproductive cycle of *P. merguiensis*, which is important for establishing appropriate fishing seasons, protecting spawning stocks, and supporting sustainable resource management. In hatchery and captive breeding programs, information on ovarian maturation can be used to accurately select mature broodstock for mating and spawning induction, thereby improving fertilisation success and larval production.

MATERIALS AND METHODS

Sample Collection

Healthy gravid female *P. merguiensis* specimens were collected from the estuarine area of Kuala Muara, Pulau Sayak, and conditioned in a holding tank at the Department of

Fisheries (DOF) Pulau Sayak hatchery prior to the experiment. A total of 50 female samples (mature females weighing 25-35 g with a total length greater than 14 cm) were collected from the sampling site during low tide between June and August 2025. During collection, water temperature, salinity and dissolved oxygen were 28-30 °C, 28-30 ppt, and 6 mg/L, respectively. Samples were then subjected to physical observation, followed by dissection and histological assessment. Mature female selection was based on the presence of visible ovaries located dorsally to the cephalothorax. When the ovaries were not clearly visible, females were identified by the presence of the thelycum located between the fourth and fifth pereopods (walking legs), which serves as the reproductive structure in female penaeid shrimp (Moraes et al., 2025). All females were subsequently placed in ice-filled containers to immobilise them before dissection. Dissection began by carefully removing the exoskeleton behind the carapace, proceeding toward the telson. All procedures involving animal handling were followed by research ethics guidelines from the Office of Research Management (RMO), Universiti Malaysia Terengganu (UMT), Kuala Terengganu, for the care and use of animals in this whole experiment as outlined in the Research Ethics Guidelines for the Ethics of Research on Animals 2014 (UMT; https://studylib.net/doc/8738550/research-ethics-guidelines—pusat-pengurusan-penyelidikan).

Gonadosomatic Index (GSI)

Body weight and ovary weight were measured and recorded to determine the gonadosomatic index (GSI). The GSI represents the relative size of the gonads in proportion to the total body weight. It was calculated using the following formula in Equation 1:

GSI: [ovary weight (g)/ total weight (g)] * 100 (Aaqillah-Amr et al., 2022) [1]

Histology

Ovarian tissues were immediately fixed in Davidson's fixative at a tissue-to-fixative ratio of 1:10 (w/v) for 24 h at room temperature, followed by dehydration in 70% ethanol overnight. The dehydrated samples subsequently underwent tissue processing through a graded ethanol series of 70%, 90%, 95%, and 100% ethanol for 2, 1, 1, and 4 h, respectively, followed by clearing in xylene for 6 hours. The samples were then infiltrated with paraffin wax at 60 °C for 4 hours prior to embedding. Embedded tissues were sectioned at a thickness of 5 µm using a rotary microtome. The sections were dried on a hot plate at 40 °C overnight and subsequently stained with hematoxylin and eosin (H&E) for 10 and 3 minutes, respectively, following the standard staining protocol described by Fischer et al. (2008).

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 26. Data for gonadosomatic index (GSI) and oocyte diameter were expressed as mean ± standard deviation (SD). Normality and homogeneity of variance were evaluated using the Shapiro-Wilk and Levene's tests, respectively. Differences in GSI and oocyte diameter among ovarian maturation stages were analysed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test for pairwise comparisons when significant differences were detected. Statistical significance was accepted at $P < 0.05$.

RESULTS

External Morphology

Table 1 illustrates the external morphology of the ovarian development stages in *P. merguiensis*. Ovary colour progressed from translucent (Stage 1), yellowish (Stage 2), light green (Stage 3), green (Stage 4), to whitish (Stage 5). The gonadosomatic index (GSI) increased significantly from Stage 1 (6.32 ± 0.80), Stage 2 (10.96 ± 1.20), Stage 3 (13.32 ± 1.68), to Stage 4 (19.70 ± 1.27), before decreasing significantly at Stage 5 (7.22 ± 0.68), with $p < 0.05$.

Histological Observation

Table 1 and Figure 1 indicated the histology of the ovarian development stages in *P. merguiensis*. Table 1 showed mean oocyte diameter increased from Stage 1 (39.4 ± 9.42 µm), Stage 2 (76.19 ± 8.89 µm), Stage 3 (134.04 ± 15.89 µm) to Stage 4 (152.04 ± 13.1 µm), then decreased during the spent stage (48.34 ± 21.9 µm), with Figure 1 illustrated Stage 1 and Stage 5 were characterised by the presence of perinucleolar $p < 0.05$. oocytes, Stage 2 by the appearance of yolkless oocytes, Stages 3 and 4 by the presence of yolky oocytes, with Stage 4 additionally marked by the presence of elongated cortical bodies.

DISCUSSION

The five ovarian developmental stages identified in this study are consistent with those reported for the same species, *P. merguiensis,* by Ayub and Ahmed (2002). Manan et al. (2022) reported similar ovary stages from Stage 1 to Stage 4 but did not observe the spent stage. Similarly, other penaeid shrimp species such as the Indian white prawn, *P. indicus* (Amanat and Qureshi, 2011), redtail shrimp, *P. penicillatus*, jinga shrimp, *Metapenaeus affinis*, kiddi shrimp, *P. stylifera* (Ayub & Ahmed, 2002), giant freshwater prawn, *M. rosenbergii* (Revathi et al., 2012), and white-leg shrimp, *P. vannamei* (Li et al., 2022) have

Table 1
External morphology and histology of banana shrimp, P. merguiensis *ovaries*

Stage	External Morphology		Histology	
	Description	GSI	Description	Oocyte size (mm)
Stage 1 (Immature)	The ovary was difficult to observe physically. It appeared as a straight, thin line along the abdomen and was clear, transparent, or translucent in colour.	6.32 ± 0.80[a]	Perinucleolar oocytes were abundantly present in the ovary	39.43 ± 9.42[a]
Stage 2 (Premature)	A more developed ovary was observed. It appeared as parallel lobes along the abdomen and was yellowish-white in colour.	10.96 ± 1.20[b]	Perinucleolar oocytes and yolkless oocytes were identified in the ovary, with an increase in nucleus size while reducing cytoplasm volume.	76.19 ± 8.89[b]
Stage 3 (Mature)	The ovary occupied a significant portion of the abdominal cavity, including the stomach in the cephalothorax region. Its colour was light green	13.32 ± 1.68[c]	Yolky oocytes were more abundant than yolkless oocytes. Late-stage yolk globules were identified, and the cytoplasm volume had increased.	134.04 ± 15.89[c]
Stage 4 (Fully mature)	The ovary filled the entire abdomen and cephalothorax. Its colour was bright green, but slightly darker compared to the previous stage.	19.70 ± 1.27 [d]	Abundant yolky oocytes with elongated cortical rods and yolk globules were identified.	152.04 ± 13.08[d]
Stage 5 (Spent)	The ovary size was shrinking but remained larger than in Stage 1. Its colour was pale cream or whitish.	7.22 ± 0.68[a]	Perinucleolar oocytes were present in fair numbers, along with follicle cells and chromatin nucleolar oocytes. Atretic oocytes were also present, undergoing reabsorption	48.34 ± 21.85[a]

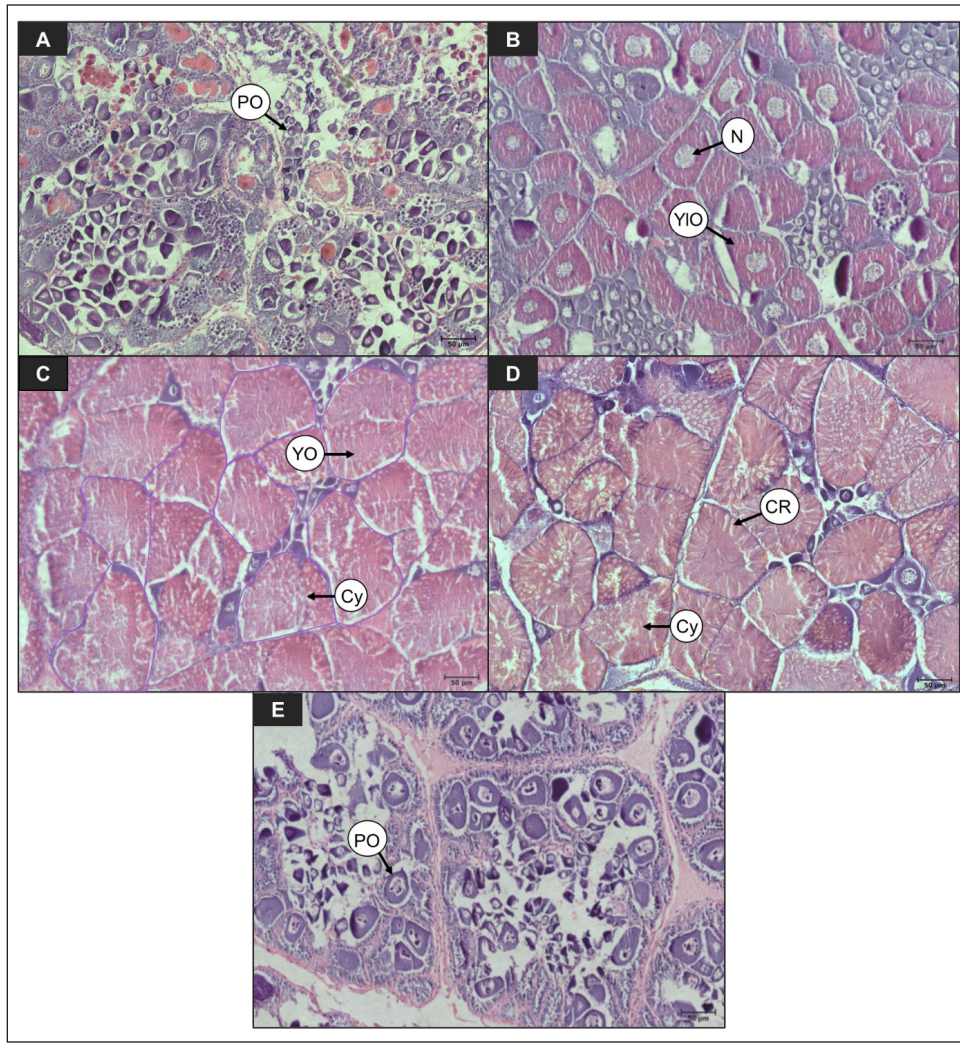


Figure 1. Ovary maturation stages of *P. merguiensis*: (A) immature, (B) premature, (C) mature, (D) fully mature, (E) spent stage. Histological features include (Cy) cytoplasm, (CR) cortical rod, (N) nucleus, (PO) perinucleolar oocytes, (YlO) yolkless oocytes, and (YO) yolk oocytes

also been classified into five ovarian stages. When compared to the present study, these species show a similar pattern of ovary colour progression, starting from translucent in early stages, turning yellowish, and reaching peak maturation with dark green colouration, followed by a whitish colour at the spent stage.

During the early ovarian development stage (Stage 1), perinucleolar oocytes dominate the ovary due to the lack of yolk. Yolk accumulation begins in Stage 2 as oocyte size increases; however, this stage is still dominated by yolkless oocytes with relatively large nuclei. In Stage 3, the nucleus was reduced and became faint, while yolky oocytes became

more abundant than yolkless oocytes, resulting in an increase in oocyte size. In Stage 4, yolky oocytes dominated the entire ovary, with no visible nucleus and larger oocyte size than in Stage 3. Stage 4 was considered fully mature and represented the optimal nutrient supply for embryonic development in shrimp eggs. In stage 5, the GSI decreases because most mature oocytes and yolk reserves have already been released during spawning. All these processes are collectively referred to as vitellogenesis, which involves the accumulation of yolk within the ovary. The yolk consists of nutrients such as proteins and lipids, specifically vitellin, a type of lipoprotein (an apolipoprotein that binds lipids), which may be synthesised either in the ovary, the hepatopancreas, or both (Revathi et al., 2012). This mechanism is supported by Li et al., who reported the expression of several lipoprotein-related genes, including Vg, ApoD, BGBP, CP, VgR, VLDLR, and LDLR-RP1, in the hepatopancreatic and ovarian transcriptomes of white leg shrimp, *Litopenaeus vannamei* during ovarian maturation. The lipoproteins from the hepatopancreas are transported to the ovary via haemolymph, as shown by Revathi et al. (2012), who observed increased lipoprotein levels in the hemolymph with advancing ovarian stages in the giant freshwater prawn, *M. rosenbergii*. The vitellogenesis process enlarges oocyte size at the end of the ovarian stage. Similar histological evidence of vitellogenesis has also been reported in other crustacean species, including the mud crab, *Scylla olivacea* (Hidir et al., 2018), blue swimming crab, *Portunus pelagicus* (Ravi et al., 2012), giant freshwater prawn, *M. rosenbergii* (Revathi et al., 2012), white leg shrimp, *P. vannamei* (Quesada-Avila et al., 2024), and red swamp crayfish, *Procambarus clarkii* (Lu et al., 2020).

Vitellin is also responsible for the colour changes in the ovary, which becomes yellowish and green in the fully mature stage. Ovary colour is further influenced by esterified carotenoids, complexes formed between carotenoids and lipids, including β-carotene, zeaxanthin, and astaxanthin, which contribute to the pigmentation of the oocytes (Li et al., 2022). Additionally, cortical rods were clearly observed in Stage 4 ovaries, indicating the completion of oocyte maturation in penaeid shrimp. Cortical rods are glycoprotein-rich structures that develop at the final stage of vitellogenesis and are arranged around the periphery of mature oocytes (Kruevaisayawan et al., 2010). Upon spawning and fertilisation, these cortical materials are released through the cortical reaction, forming a jelly-like protective envelope surrounding the egg (Subramoniam, 2017). This envelope functions as a barrier that helps prevent polyspermy, microbial invasion, and environmental stress during early embryonic development, particularly in externally fertilised crustaceans such as penaeid shrimp (Subramoniam, 2017).

CONCLUSION

There are five stages of ovarian maturation in *P. merguiensis*: immature, premature, mature, fully mature, and spent. These stages are differentiated based on ovary colour,

gonadosomatic index (GSI), and histological assessment, including the presence of perinucleolar oocytes, yolkless oocytes, yolky oocytes, and cortical rods. In the future, information regarding ovarian developmental stages in *P. merguiensis* may contribute to a better understanding of the spawning season and reproductive cycle of this species, which could support the establishment of appropriate fishing seasons, protection of spawning stocks, and sustainable fisheries management. Likewise, information on ovarian maturation may be further applied in hatchery and captive breeding programs for accurate broodstock selection, spawning induction, and improvement of fertilisation success and larval production.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

All data supporting the findings of this study are included within the paper. No additional data are available.

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Life Cycle Assessment of Conventional Broiler Farm System in Malaysia and Carbon Footprint Mitigating Strategies

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ABSTRACT

The livestock industry is a significant anthropogenic source of greenhouse gas (GHG) emissions due to rapid development and intensified production systems. This study evaluated GHG emissions from a commercial broiler production system using a life cycle assessment (LCA) approach from cradle to farm-gate and assessed carbon footprint mitigation strategies for poultry litter (PL) management. Three scenarios were compared: selling PL (farmer's practice), composting, and conversion into biochar. The LCA model was developed in accordance with Intergovernmental Panel on Climate Change (IPCC) guidelines and aligned with national sustainability goals. From each production cycle, 63.97 tCO_2-eq were estimated per house, with the largest contributor from feed production, followed by energy expenditure. Carbon reduction was reported to be 25.79%, 13.21%, and 16.98% by selling PL, composting, and biochar conversion, respectively. In carbon *hotspot* analysis, feed processing and energy consumption were determined as the major contributors from the production. Although selling PL brought greater carbon mitigation, it shows the least sustainable waste management and environmental gains compared to compost and biochar. Future studies should assess the emissions from land-use change, composting, and biochar production to improve the accuracy of carbon footprint estimations. These findings provide a clearer guide to improve poultry waste management and encourage achievement of national net-zero emission goals.

Keywords: Broiler production, cradle-to-farm gate, GHG emissions, LCA, mitigations, Malaysia

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INTRODUCTION

Greenhouse gas (GHG) emissions and climate change have been a long-term global challenge. Malaysia's livestock sector accounts for approximately 4% of the total agricultural Gross Domestic Product (GDP) in 2024 (Ministry of Economy, 2025).

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The agrifood system was reported to contribute approximately one-third of global anthropogenic GHG emissions, dominated by the livestock sector (FAO, 2022). Attia et al. (2024) reported that almost 10% of the total livestock emissions stemmed from the poultry sector, and a continuous increasing trend was discovered in recent years by Hegarty et al. (2024). According to OECD (2025), Malaysia's poultry industry is rapidly growing, with a forecasted average annual growth rate of 1.5% for 8 years due to high demand for poultry products. Based on the 2019 List of myGAP Poultry Farms by the Department of Veterinary Services Malaysia (DVS), they listed over 400 chicken farm operations were registered, while actual numbers are expected to be higher due to the presence of unregistered smallholder farmers. Regardless of its importance, GHG emissions records from Malaysia's livestock industry, particularly from the poultry sector, remain scarce. The majority of existing sources relied on Tier 1 assessment, using default values from global datasets such as IPCC and EPA. Approximately one-third of methane (CH_4) and 65% of nitrous oxide (N_2O) from the livestock total emissions were derived from poultry farming, dominated by manure management (Zubir et al., 2022). This emphasises that more comprehensive and localised studies should be done for efficient mitigation strategies.

Life Cycle Assessment (LCA) is a systematic, formalised methodology used to evaluate the environmental impacts and resource management of a product or process throughout its life cycle (Malaysian Plastics Forum, 2018). Malaysia has been introducing LCA for years to promote green evolution in the Ninth Malaysia Plan 2006-2010 (2006), yet market adoption was only observed in recent years.

Compliant with international agreements such as the Kyoto Protocol (1997) and the Paris Agreement (2015), much research in Malaysia has emerged as LCA-based studies to estimate GHG emissions across various sectors. For instance, a study conducted on the certified sustainable practices of oil palm plantations have reported a more than 10% of carbon mitigation efficiency (Bok et al., 2022); in crop production, such as paddy, Nurfarhain Mohamed Rusli et al. (2023) identified emission hotspots were preponderance from early plantation (Scope 1 emissions).

Nevertheless, LCA adoption in Malaysia is still relatively low, as several obstacles were identified, such as insufficient funding, specialists, and promotional efforts (Hafizan et al., 2021). Furthermore, there is a knowledge gap among local industries, even with the internationally published standards (Rajendran et al., 2021). In fact, this led to a disconnect between Malaysian industries' operations and LCA, resulting in a shortage of reliable national data for better assessment accuracy.

High climate action commitment was demonstrated by Malaysia through various national policies. In 2023, the National Energy Transition Roadmap (NETR) was released, aiming to achieve net-zero emissions by 2050. One of the major incidents resulting in this action was due to a drastic increase in net emissions of more than 200% since 1990, reported

by the National Climate Change Policy 2.0 (NRECC, 2022), which prompted greater public concerns about the climate change impacts on agrifood systems. In the updated Malaysia First Nationally Determined Contribution (NDC) submitted to UNFCC (2021), agriculture and waste sectors were restated to play one of the key roles in achieving 45% total carbon mitigation intensity by 2023. Additionally, in early 2013, the MYCarbon Programme involved 26 listed companies that participated in voluntary carbon reporting and reduced their carbon emissions, showing encouragement to the local industries.

Poultry litter (PL) acts as one of the major pollution sources in poultry farming, with high nutrient-mix content, specifically droppings, feathers, and feed waste (Maruthamuthu et al., 2024). It is a common poultry industry by-product, consisting of feed residues, bedding materials, feathers, and excreta. PL has been widely used for centuries as an organic fertiliser due to its high nutrient content and traditional utilisation through direct application to agricultural land. Several studies have demonstrated its positive impacts on soil fertility and nutrient improvement (Mirabello et al., 2025); still, the adverse effects of the traditional practices are worrisome.

In poultry production, the majority of GHG emissions stemmed from manure management, regardless of production or handling processes (Meda et al., 2011). Moore et al. (2025) reported a significant increase in GHG emissions from 4-week to 8-week age flocks, likely due to larger manure production as the birds grow. This could also be explained by its slow decomposition and lower enzymatic properties compared to composted biodegradable materials, resulting in accumulation of GHGs within the poultry houses. Exacerbating this issue, large quantities of PL generated daily in intensive poultry farming systems have not been managed in a proper manner, leading to negative consequences for the local community.

Proper waste management is crucial in minimising environmental impacts. Some promising approaches are through composting or transforming into biochar. Composting is a biological process involving the degradation of organic matter into a nutrient-rich product through the elevation of temperature within the system under natural aerobic conditions (Nurin et al., 2024). Through composting, almost 50% of poultry waste could be reduced, introducing lower space requirements for storage and disposal.

Composting is globally recognised for its contribution towards environmental benefits. It is known for its high potential in GHG mitigation, tackling climate change, and serving as a soil amendment, thereby improving crop yield and soil water-holding capacity (Nurin et al., 2025). Additionally, certain antibiotic-resistant genes have been shown to be eliminated under specific composting conditions (Xiong et al., 2025), further supporting composting as an effective and sustainable waste treatment option. In the poultry industry, composting is a particularly attractive method for waste management due to its simplicity and cost-effectiveness. Aerobic composting is among the most common techniques used

to treat poultry manure (Ma et al., 2025). Composting is a process to produce stable organic matter (OM) by fostering microbial activities under controlled conditions, i.e. temperature, moisture, and oxygen level.

However, several studies have reported that composting can also have negative environmental impacts, primarily due to the emission of major GHGs such as CH_4, CO_2, and N_2O, which are associated with various bacterial genera during anaerobic phases of the process (Cui et al., 2025). Gu et al. (2024) reported an increasing trend of CH_4 and carbon dioxide (CO_2) emissions during the anaerobic phase of composting due to methanogenic microbial activity. In contrast, N_2O concentrations tend to rise during the intermediate phase as a result of incomplete oxidation of ammonia, as illustrated in Figure 1. An overall reduction in sustainability practices might be observed from the accumulation of GHGs throughout these stages.

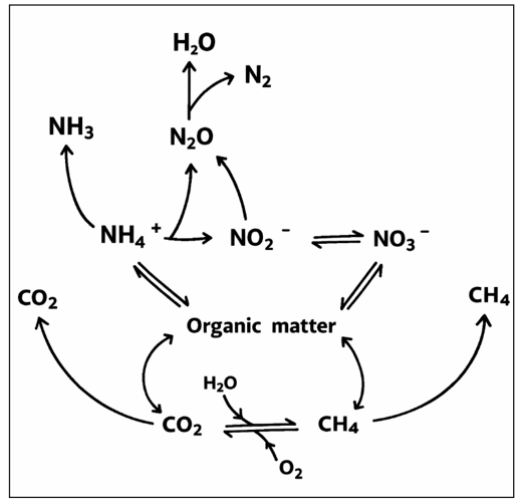


Figure 1. Production of GHG (i.e. CH_4, CO_2, and N_2O) during composting [Cui et al., 2025]

As an alternative to composting, biochar has recently gained significant attention due to its economic and environmental advantages. Biochar is a stable carbonaceous compound with the capability to improve soil health and carbon mitigation, a compliant solution for the circular economy in agriculture (Weng & Cowie, 2025). On top of it, biochar could enhance plant adaptation to climate change challenges, improving their resilience towards environmental stress, particularly during elevated temperature scenarios. It helps regulate interspecific relationships and improve crop production even under unfavourable medium conditions (Laungani et al., 2025). These extraordinary properties establish biochar as a rising star in promoting carbon mitigation and play an important role in building a sustainable future.

In response to the demand for national data and sustainable agricultural practices, this study aims to quantify total GHG emissions produced from commercial broiler production in Malaysia from cradle to farm gate and identify the emission *hotspots*. Furthermore, the study evaluates the net carbon footprint produced from three different manure treatments: 1) selling PL as raw waste, 2) composting, and 3) converting it into biochar. This research represents a crucial milestone in improving environmental assessment capabilities within Malaysia's broiler sector. By incorporating local data and applying advanced LCA methodologies, the study supports national climate goals and promotes more sustainable livestock production practices.

MATERIALS AND METHODS

Broiler Production Description

This study was conducted in an intensified commercial broiler farm located at Kulai, Johor, Malaysia. Three closed-house poultry houses were used for assessment. A minimum of 20,000 Ross 308 broiler chickens were raised per house to maximise the production profit. As the farm practices animal welfare standards, chicks were initially grown at a stocking density of 0.1 sqft/bird, and gradually increased to a minimum of 0.8 sqft/bird at the market age.

Day-old-chicks (DOC) from the breeder farm were transported to the broiler farm for growth within 24 hours after hatching. Through feather sexing in the hatchery, the chicks were packed separately according to their sex and transported to the broiler farm, where they generally have a proportion of 60% female and 40% male chicks.

The broiler growing process takes approximately 42 days, and an additional 20 days for thorough cleaning and disinfection, and preparing for the next cycle intake. The whole production process takes an average of 62 days to complete. From DOCs to an age of 21 days, the chicks were fed with starter feed and were gradually replaced by grower feed at the age of 21 till market weight, ranging from 2 to 2.5 kilograms, which aligns with the data provided by the Department of Veterinary Science Malaysia (DVS). Both types of feed were supplied by FFM Grains and Mills Sdn Bhd, located in Johor Bahru, Malaysia. Supplementation, i.e., probiotics and plant extract products, was used to treat the chickens to enhance their immunity and control disease outbreaks. The supplements used were supplied by a biotechnology company located in Johor Bahru.

Sawdust, with an important function as a bedding material, was transported to the farm at least two days before the DOCs' arrival. Insecticides were constantly bought to the farm for cleaning and disinfection purposes, regardless of the presence or absence of chickens.

Life Cycle Impact Assessment (LCIA)

The study focused on global warming potential (GWP_{100}) resulting from GHG emissions. According to the Climate Change Sixth Assessment Report IPCC (2023), the GWP_{100} values were listed in Table 1. CO_2 is the reference for estimating GWP_{100}: it remains the value "1" regardless of time period. CH_4, as one of the major ozone destructive gases, contributes approximately 29 to 30 GWP_{100}, followed by N_2O with 273 times higher heat absorption and remaining in the earth, denoting significant global warming effects (U.S. EPA, 2025).

Table 1
100-year Global Warming Potential (GWP_{100}) of CO_2, CH_4 emitted from combustion of fossil fuel and non-fossil fuel sources, and N_2O

Components	[1]GWP_{100}
CO_2	1
CH_4- fossil	27.9
CH_4- non-fossil	29.8
N_2O	273

Note. [1]Climate Change Sixth Assessment Report IPCC (2023)

Poultry Greenhouse Accounting Framework (Po-GAF) V1.45, originated from Primary Industries Climate Challenges Centre (PICCC), was modified to align with the farm's conditions. In this study, CO_2, CH_4, and N_2O were primarily estimated using the formulae provided in the PICCC model in Equation 1. The CH_4 EF was estimated using Equation 10.23 in Chapter 10: Emissions from Livestock and Manure Management (IPCC, 2019).

$$EF_M = B_0 \times 0.67 \times MCF \times AWMS \quad [1]$$

where B_0 is the maximum producing capacity with the reference value provided in Table 10.16A, 0.67 is the conversion factor, MCF is the CH_4 conversion factor listed in Table 10.17, and AWMS is the animal waste management system fraction. As the farm practices fully sell PL, the AWMS is 100%.

Data obtained from Equation 1 was then used in CH_4 production (Equation 2) whereas N_2O production from manure management was estimated using Equation 3.

$$CH_4 = N \times VS \times AWMS \times EF_M \quad [2]$$

$$N_2O = N \times NE \times AWMS \times EF_{N2O} \times \frac{44}{28} \quad [3]$$

where N is the flock population, VS is the volatile solids excretion per bird (kg VS/bird), NE is the N excretion (kg N/bird), EF_{N2O} is the nitrous oxide EF listed in Table 10.21, and $\frac{44}{28}$ is the conversion factor.

In Malaysia, electricity is provided by Tenaga Nasional Berhad (TNB). The total electricity consumption per house was quantified, and emissions were assessed using country-specific EF reported in NRECC (2022) Table B-1. For emissions from stationary fuel such as fuel tanks, and for mobility such as transportation, the EFs were obtained from Table A-2 and Table 13-e, respectively, from the same source as the electricity EF.

Emissions associated with feed production were estimated by aggregating the raw feed materials based on their respective countries of origin, utilising emission intensity values derived from established literature. The emission intensities for each material are presented in Table 2. Due to confidentiality constraints imposed by the feed mill, the proportional composition of individual raw materials was not disclosed.

LCA Goal and Scope

This study's LCA goal and scope were essential to clarify according to the ISO 14040/14044 standard. The objective of this LCA study was to quantify the total GHG emissions and mitigation efficiency from the broiler farm from cradle to farm gate.

Table 2
Starter and grower raw materials GHG emission intensity

Material	Origin Country	[1]Emission intensity (t CO_2-eq/t material)	Reference
Corn	Argentina	0.20	Arrieta et al. (2018)
Soybean Meal	Argentina	0.41	Parajuli et al. (2022)
Corn Gluten Meal	USA	0.98	Taylor et al. (2023)
Crude Palm Oil	Malaysia	1.17	Kulim (Malaysia) Berhad (2020)

Note. [1]Greenhouse gas emission intensity

Scope and Boundary

The system boundary comprises the cradle-to-farm gate, which includes the production inputs from DOC intake until the adult chicken was harvested. The assessment was consolidated from both starter and grower emissions, and results were expressed in one-kilogram finishing chicken and presented in kilograms of CO_2 equivalent per kilogram of live-weight (kg LW/ kgCO_2-eq) of GHG emissions.

For better calculation of the GHG emissions, following the Corporate Standard (2004), the LCA was segregated into three scopes, i.e. Scope 1 (on-farm emissions/ direct emissions), Scope 2 (purchased electricity), and Scope 3 (upstream activities). The boundary of this LCA study was illustrated in Figure 2.

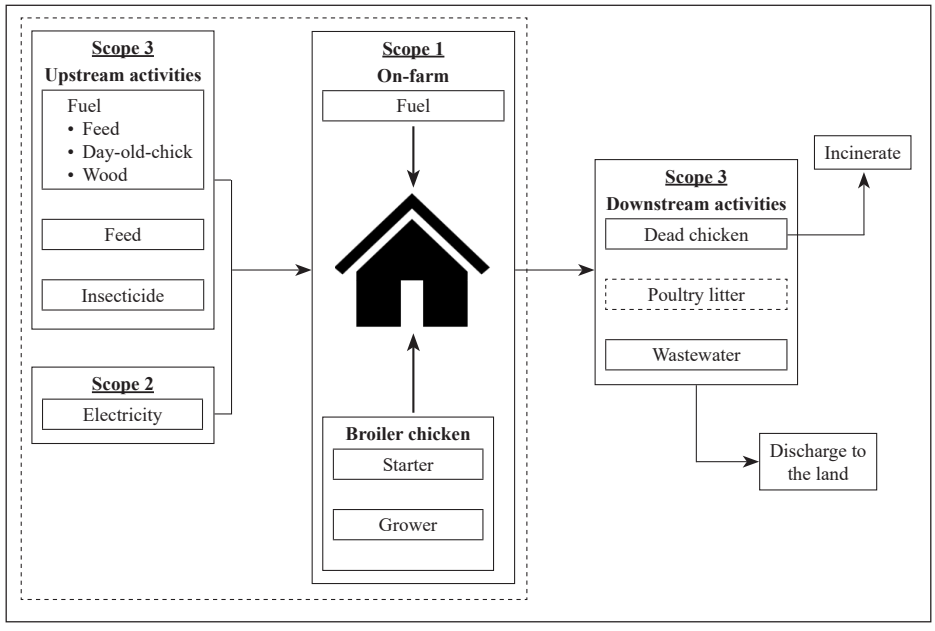


Figure 2. System boundary with activities quantified in this assessment. Subjects fall in dashes lines were in the boundary of assessment

In Scope 1, the emissions resulted from on-farm activities, specifically from manure management and fuel utilisation. Scope 2 emissions encompass electricity usage, whereas Scope 3 involves both upstream and downstream activities. For upstream activities, it involves fuel consumption for transporting DOC, feed, and wood shavings into the farm. As wood shavings and DOC were only transported into the farm once (before the cycle started), the fuel consumption was calculated as one trip; feed was transported into the farm for two times per week, and thus, the total fuel consumption was assessed based on the total frequency transported into the farm. Emissions during feed production also fell under Scope 3 as it is produced for farm use. Downstream activities involve several processes such as PL handling and other by-products handling; however, only PL management has fallen into this assessment due to limitations of data for other co-products produced.

DATA INVENTORY

The study comprises both primary and secondary data inventory for more accurate GHG emissions estimation. For a better understanding of the process flow, the operational sequence was illustrated in Figure 3.

Primary Data Inventory and Sample Collection

As defined in the Benedictine University Library (2025), primary data refers to the information that is directly collected by the researcher through methods such as surveys, interviews, or experiments. The purpose is specifically gathered for addressing and understanding the study hypothesis.

The primary data for this study were obtained mainly through on-field sample collection and laboratory analysis. PL samples were collected bi-weekly starting from DOC intake (Week 1) until harvested (Week 5), i.e., Week 1, Week 3, and Week 5. These sampling periods represent different growth stages of the broiler chickens, including early growth, intermediate growth, and finishing stages. Changes in birds' age and feed types resulted in different nutrient excretion, which influences GHG production. Therefore, collecting samples at these intervals provides a more representative estimation of total GHG emissions.

Grab samples were collected randomly from five to ten spots in each broiler house and mixed thoroughly to obtain a representative composite sample. From each house, six composite samples were collected. After the cleaning process at Week 8, three grab PL samples were collected from each house, which were obtained from the mixture of five to ten arbitrary points. All solid samples were immediately stored in an ice box before being transferred to an Acson R134a freezer (-18 °C) for further analysis. Total solids (TS) and volatile solids (VS) content were analysed following the procedures outlined by MacLeod et al. (2013), employing the methodologies recommended in the *Second Edition of Recommended Methods of Manure Analysis* (Wilson et al., 2022). Each sample was analysed in triplicate to determine the average values.

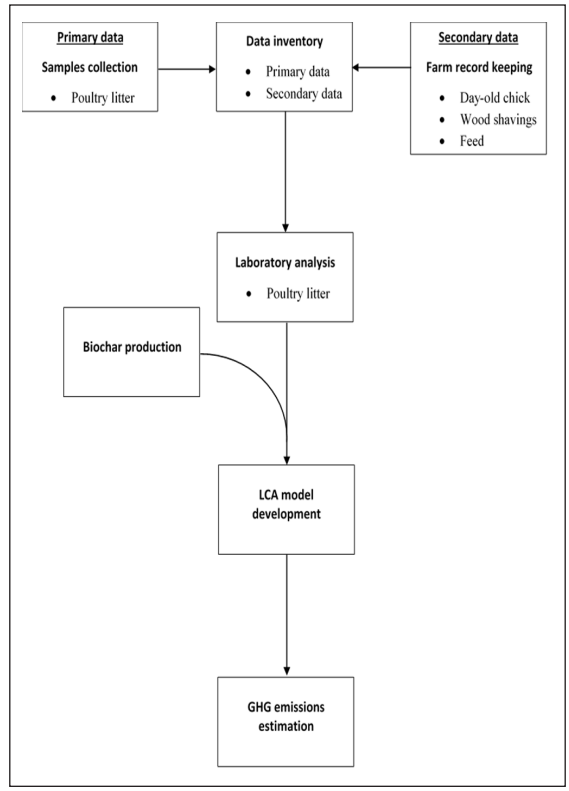


Figure 3. Process flow of the LCA

As the farm does not practice wastewater treatment, the impacts and downstream emissions from the discharge activity were excluded in this assessment.

Secondary Data Inventory

Secondary data refers to historical data generated by the organisation or institutions as part of their routine record-keeping. This data is later retrieved and utilised from various existing datasets for research or analysis purposes (Wagh, 2025). The data collected were flock population each house, total feed consumption, daily body weight, total insecticides purchased, total electricity consumption, and the total broilers marketed and coproducts produced at the farm gate. The feed composition was shown in Table 3, encompassing the farm's feed management, i.e. starter and grower feed.

Table 3
Feed nutrient compositions of starter and grower

Composition	Starter	Grower
Dry Matter (g/kg)	885	886
Crude Protein (g/kg)	198	211
Crude Fat (g/kg)	47	82
Crude Fibre (g/kg)	23	22
Ash (g/kg)	52	48
Nitrogen-free Extractives (g/kg)	566	524
Gross Energy (kcal/kg)	3835	4066

The data were de-identified and compiled in consolidated form to protect the confidentiality of proprietary corporate information.

Handling of Co-products

Finished broiler chickens are the primary products from the farm, while PL, bird carcasses, and wastewater generated during cleaning processes are considered as secondary outputs. The predominant method for managing PL involved selling it to local crop production enterprises. Emissions resulting from land application of PL were excluded from the assessment, as they fell outside the defined system boundaries. Bird carcasses generated during the production cycle were disposed of through incineration; however, the associated emissions were not evaluated due to a lack of reliable data. Wastewater produced during cleaning activities was discharged into the soil, and its environmental impacts were not included in this assessment.

Mitigation

After each production cycle, the farm practised selling PL as fertilisers to other crop production, which is one of the main co-product handling methods. However, due to potential adverse effects of PL on soil properties, including pH change, organic matter content, concentration of micro- and macronutrients, eluviation, and crop yield (Silva et al., 2025), alternative mitigation strategies have to be developed. Also, mass antibiotic usage in poultry farming has raised environmental and public health concerns. Lopes et al. (2024) have discovered multi-antibiotic bacterial strains in PL, and they exhibit great potential in horizontal gene transfer of antimicrobial resistance genes, especially in agricultural soil, consequently posing both environmental and human health risks. In intensive monoculture cropping systems and similar livestock populations, the environmental persistence and adaptation of pathogenic microorganisms potentially increase the reinfection of existing host populations (Wesley et al., 2000). This created concerns not only on the GHGs emissions, also to the public health. To improve the mitigation system, the study proposed composting and converting PL into biochar. Both mitigation methods were calculated for their total carbon sequestration.

Poultry Litter

The total amount of PL generated during the cycle in this study was recorded and assessed. Modelling the actual situation of the farm management, all the PL was sold to other crop production as fertilisers. No litter was recycled for the next production cycle.

Composting

Composting is a biological process that converts biomass into nutrient-rich organic matter through several successive stages. The process begins with the degradation of organic materials by mesophilic microorganisms (mesophilic phase), followed by the thermophilic phase, during which microbial activity intensifies, and heat is released as a by-product of decomposition. Finally, the curing phase occurs, characterised by the stabilisation and maturation of the remaining organic matter (Lim et al., 2019).

To estimate the total mitigation potential of composting in this study, it was assumed that all PL were composted, with animal waste management allocated entirely (100%) to composting. As the study does not have primary data obtained from the PL compost from the farm, reference values were retrieved from Naumova et al. (2024) and Tiquia & Tam (2000), where the characteristics of PL samples were more similar to this study (Table 4).

Biochar Production

Biochar is a stable, carbon-rich material derived from biomass sources such as wood chips, manure, and agricultural residues and is primarily used for carbon sequestration (USDA, 2025).

Biochar could be produced mainly through a few crucial steps, including organic matter collection, drying, grinding, and sieving before thermal processing, which would be carried out under minimal or absence of oxygen, named pyrolysis or carbonisation processes. For improving adsorption capacity, post-treatment is often done to enhance char's surface area. Optimal production generally occurs at temperatures above 200 °C (Xiang et al., 2020). Pyrolysis refers to the thermal decomposition of biomass into a carbonaceous product under limited or no oxygen conditions, usually at temperatures below 900 °C. The resulting biochar possesses a porous structure that enhances water-holding capacity, nutrient retention, pH modification, and cation exchange capacity (Zakaria et al., 2023).

In this study, after the collection of PL at Week 8 of production, 20 g of PL samples were used to convert into biochar, referring to the methods conducted by Chin et al. (2024). Biochar was first pre-dried in a Memmert Universal Lab Oven at 60 °C for 12 hours. After drying, the samples were ground and crushed into 0.20 mm particle sizes and further dried in an oven at 105 °C for 24 hours to minimise the water content before pyrolysis. Following their method, the samples were pyrolysed at 420 °C for 30 minutes in an ELF 11/14B Chamber Furnace to maximise the organic carbon recovery from PL. The dry weight was determined by heating it at 105 °C for 24 hours (Nkomo et al., 2021). Biochar yield was calculated as a percentage, as shown in Equation 4.

$$\text{Biochar yield} = \frac{\text{Biochar produced, g}}{\text{Biomass pyrolysed, g}} \times 100\% \quad [4]$$

Table 4
Poultry litter (PL), compost, and biochar composition obtained from primary data, except for composting, which used reference values

	DM	Moisture	Ash	Carbon	Organic Carbon	Yield
			%			
PL	71.596	28.404	89.935	41.1	-	-
*Compost	75.5	24.5	10.65	20.45	9.425	-
Biochar	99.971	0.029	-	44.86	33.73	61.885

Note. (*) indicated data obtained from other sources, i.e. Naumova et al. (2024) and Tiquia & Tam (2000); DM, dry matter content on dry basis; Yield, total yield after treatment

Carbon Sequestration

Equation 5 shows the method for carbon sequestration assessment using PL and compost, while Equation 6 is for biochar, where $\frac{44}{12}$ is the conversion factor to convert the carbon reservoir in the biomass into CO2, Ydm is the biochar yield in dry mass, and C-stock is the carbon stock, adapted from Li et al. (2021) and Suhardono et al. (2024). The results were accounted in t CO2-eq.

$$CO_2 \text{ sequestration} = \text{Carbon-stock (t)} \times \frac{44}{12} \quad [5]$$

$$CO_2 \text{ sequestration}_{\text{-Biochar}} = \text{Carbon-stock (t)} \times Y_{dm} \times \frac{44}{12} \quad [6]$$

C-stock is defined as the reservoir or ecosystem which has the ability to hold or release carbon, such as soil (Newell-Price et al., 2023). To determine the C-stock in PL, compost, and biochar, they are calculated using Equation 7 (Muthmainnah et al., 2021) and Equation 8 (Goslee et al., 2015), respectively. Total carbon content (C%) was quantified using a Carbon Nitrogen Sulphur (CNS) elemental analyser, following *628 Series: Elemental Analysis* by Combustion (2016).

$$\text{C-stock}_{\text{-PL/Composting}} \text{ (t)} = \text{Dry mass of sample (t)} \times \text{C\%} \quad [7]$$

$$\text{C-stock}_{\text{-Biochar}} \text{ (t)} = \text{Dry mass of feedstock (t)} \times \text{C\%} \quad [8]$$

Emissions from biochar production were excluded from the assessment due to insufficient data.

Exception

Emissions resulting from the disposal of bird carcasses were excluded from this study due to nondisclosure by the farm. Furthermore, carbon emissions associated with wood

shavings, insecticide use, and dietary supplementation during broiler production were omitted owing to insufficient data availability. Additionally, emissions during biochar production were excluded from the study due to insufficient data. Lastly, land-use change (LUC) was considered outside the defined system boundaries of the LCA.

Data Analysis

A one-way Analysis of Variance (ANOVA) was performed to evaluate significant differences in TS and VS of PL across different weeks. All statistical analyses were conducted at a 95% confidence level. Tukey's post-hoc test was applied for multiple mean comparisons.

RESULTS AND DISCUSSION

Broiler Performance

Based on the performance data presented in Table 5, the average body weight observed was lower at hatch (Day 0), but it was consistently higher than the values reported in the Ross 308 Performance Handbook (Aviagen, 2022) from Days 7-14. However, a slower growth in body weight was observed after Day 14. This discrepancy may be attributed to the findings of Cowieson and Parsons (2024), who reported that reduced feed intake in smaller chicks leads to diminished growth performance. This observation is consistent with the present study, where the reduction in feed intake observed at Day 14 may have contributed to the subsequent decline in body weight gain.

Table 5
Broiler performance from day-old chick to market weight

Age (day)	ABW (g/bird)	Cumulative Feed Intake (g/bird)	ABWG (g/bird)	FCR
0	38.67	-	-	-
7	266.83	167.31	228.17	0.73
14	611.00	502.08	572.33	0.88
21	980.67	1039.38	942.00	1.10
28	1503.33	1732.61	1464.67	1.18
35	2050.00	2686.43	2011.33	1.34
Market	2540.00	3397.84	2501.33	1.36

Note. ABW, average body weight; ABWG, average body weight gain with Day 0 as the baseline body weight; FCR, cumulative feed conversion ratio

On Day 0, the average body weight of the chicks was considerably lower than the reference value reported in *Ross 308 Performance Handbook* (44 g). However, the average body weight exceeded the recommended standard at Weeks 1 and 2, despite a similar and

lower cumulative feed intake, respectively. At Day 7, cumulative feed intake was slightly higher than the standard (166 g/bird), which might be due to lower starter feed nutrient density (Lamot et al., 2019) or a compensatory feed consumption behaviour as described by Morais et al. (2017). Post-hatch chicks may exhibit increased feed consumption to compensate for insufficient nutrient intake and support rapid development of bone and muscles.

The lower FCR values from Day 7 (0.73) to 14 (0.88) were likely influenced by the averaging of data from the three poultry houses, which consisted of a mixed population of small chicks (≤37 g) and medium-sized chicks (>37 g), resulted in slightly higher calculated average body weight while maintaining relatively lower feed intake, resulting in an artificially improved FCR during the early growth development. After Day 21, the introduction of grower feed may have enhanced the growth performance of small-sized chicks, allowing them to undergo compensatory growth and achieve similar body weight and feed intake as the medium-sized chickens. Consequently, the FCR values increased and followed the more expected trend as the flock progressed through the later growth stages.

Nevertheless, the growth performance after Day 14 gradually declined along with reduced feed intake. Although Khulel et al. (2020) reported that smaller chicks could negatively affect body weight gain and feed intake, this factor is unlikely to be the sole contributor in the present study, as the flock demonstrated exceptional body weight gain during the first two weeks of production. Poor flock uniformity was expected to be one of the contributors to this phenomenon, especially starting from harvesting, which leaves smaller or stunted-growth birds behind the catch. As catching operations act as stressors to the birds, inducing fear, agitation, and abnormal behaviour (Wolff et al., 2019), feed intake and growth rate could be disrupted, eventually reducing body weight gain.

Emissions by Scopes

As illustrated in Figure 4, Scope 3 emissions constituted the largest proportion of total GHG emissions (37.18 tCO_2-eq) associated with conventional broiler production from cradle to farm gate, followed by Scope 1 (15.01 tCO_2-eq) and Scope 2 emissions (11.78 tCO_2-eq). Scope 3 emissions primarily stemmed from upstream processes, including feed production, insecticide, and fuel consumption for the transportation of materials to the farm.

Throughout the broiler farm production, the farm emitted a total of 25.03 tCO_2-eq GHG solely from the feed production, comprising 67% of the Scope 3 emissions, followed by 33% of emissions from fuel, and 0.3% from insecticide. This suggests that the majority of emissions within the broiler production system originate not from on-farm activities but rather from external upstream sources. Indeed, the cost of broiler production in Malaysia is mainly through the production inputs, mainly from feed cost (Abdurofi et al., 2017). Following Scope 1 emissions, it is attributed entirely to on-site manure management,

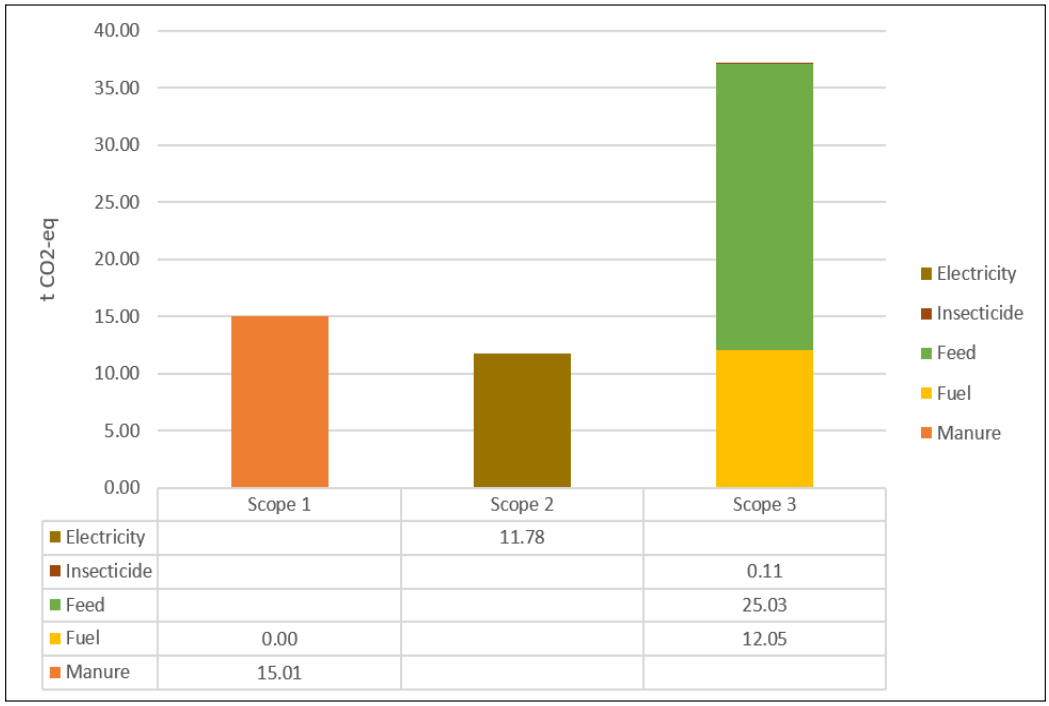


Figure 4. Total GHG emissions in the conventional broiler production farm from cradle-to-farm gate according to Scope 1, 2, and 3, with total emissions of 63.97 tCO_2-eq/house/cycle

reflecting the minimal contribution of fuel use to the farm's direct emissions, as the majority of the daily tasks were performed by hand. Though Scope 2 emissions consist solely of electricity consumption, its result is comparable to that of Scope 1, bringing attention to the environmental impacts from electricity generation.

Hotspot Analysis

Following scope-based emissions (Figure 4), Figure 5 illustrates a more in-depth evaluation of the carbon footprint incorporated with production activities. According to Figure 5, feed purchased indicated the main contributor to GHG emissions in conventional broiler production from cradle-to-farm gate, with an approximate 39% of total emissions. While manure management acted as the second contributor with 24% of total emissions, electricity and off-farm fuel consumption represented similar amounts of carbon footprints, 18% and 19%, respectively. As the farm does not practice wastewater treatment, emissions produced from the disposal were indeterminate.

In fact, fuel consumption and insecticide usage in Scope 1 and 3, respectively, were found to have negligible outcome to the overall emissions. This could be explained by low consumption of fuel usage during the brooding period, which has often been reported

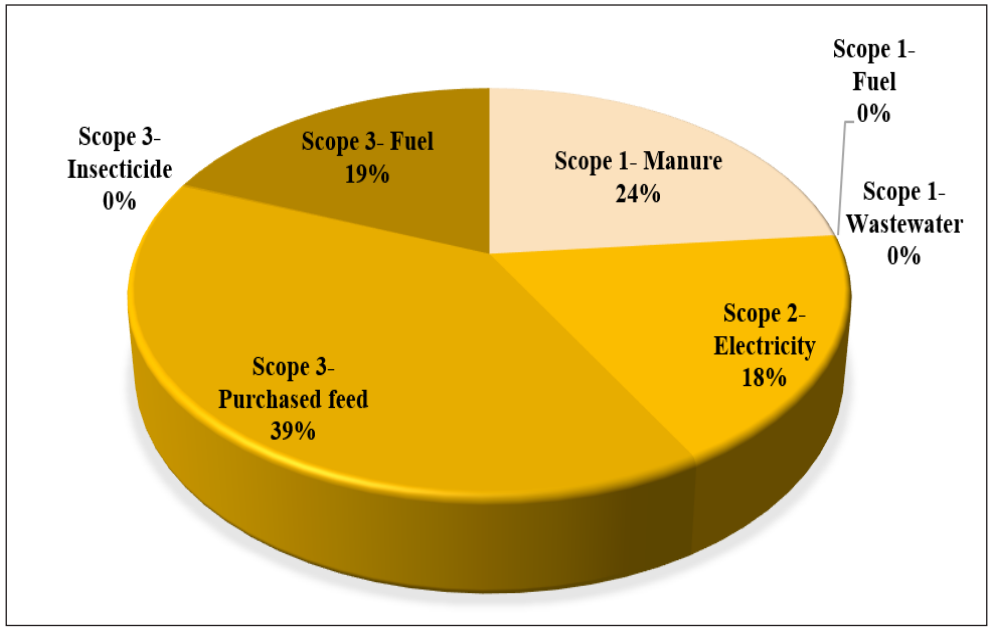


Figure 5. GHG hotspot analysis in the conventional broiler production farm from cradle-to-farm gate with a total emission of 63.97 tCO_2-eq/house/cycle

to play a key role in on-farm emissions (MacLeod et al., 2013). The analysis indirectly outlined the low dependency of the farm on fossil fuels, primarily due to the implementation of automated closed-house systems, which rely heavily on electricity for daily operations. Low emissions stemming from pesticide usage likewise indicated limited application of anthropogenic inputs, suggesting more sustainable farming practices.

Nevertheless, energy utilisation (fuel and electricity) represented a significant amount, with a total of 37% of overall emissions, which went beyond manure management, yet remained lower than feed production. This finding further highlights the profound impact of feed production on GHG production in the broiler production chain, aligning with the study by Ogino et al. (2021). By the same token, Zisis et al. (2023) concluded the main source of carbon emissions in broiler production arose from feed and LUC. Results from this study are consistent with their findings, with the highest emissions *hotspots* were associated with purchased feed. Additionally, energy used for PL and carcass disposal was identified as a key contributor to overall GHG emissions during production.

Overall Emissions and Mitigation

Table 6 represents the emission intensities for broiler production based on a cradle-to-farm gate assessment, covering three PL management options: selling untreated PL, composting, and biochar production. The baseline carbon footprint was calculated at 1.54 kg CO_2-eq/

kg LW/cycle. Among the options evaluated, selling PL showed the largest reduction in emissions, decreasing the footprint by 29.87% (0.46 kg CO_2-eq/kg LW). Biochar production followed by a reduction of 20.13% (0.31 kg CO_2-eq/kg LW), while composting resulted in a smaller decrease of 15.58% (0.24 kg CO_2-eq/kg LW).

Table 6
Summary of net greenhouse gas emissions with different mitigation methods from cradle-to-farm gate resulting from the broiler production farm

Subject	*Emission Intensity (kg CO_2-eq/kg live weight)	Total Carbon Reduction (%)
Total (without mitigation)	1.54	-
After selling poultry litter	1.08	29.87
After composting	1.30	15.58
After conversion to biochar	1.23	20.13

Note. (*) Emission intensity indicates total carbon emissions

However, the apparent benefit of selling PL needs to be interpreted carefully. The reduction is largely due to emissions being shifted across farm boundaries rather than actually reduced. In other words, untreated litter is simply transferred elsewhere, which lowers the farm-level footprint but does not solve the underlying environmental issue. This raises concerns from a sustainability perspective.

Raw PL contains high levels of N and phosphorus (P), which have high mobility in soil that is prone to leaching, water pollution, and air quality problems (Gržinić et al., 2023). The natural decomposition of untreated litter could generate CH_4, ammonia, and other volatile compounds, worsening environmental impacts. Since PL consists of animal droppings, feathers, and residual feed materials, it contains abundant nutrients, including N, P, carbon (C), and oxygen-containing compounds, which serve as substrates for microbial decomposition and subsequently contribute to greenhouse gas (GHG) emissions.

Compared to selling untreated litter, composting or conversion into biochar seems to be a more promising mitigation strategy. Composting is a method for converting biologically degradable substances into nutrient-rich soil amendments. During composting, moisture loss occurs through evaporation and degradation of water-holding organic matter in PL, resulting in a reduction of PL volume while improving the carbon-to-nitrogen ratio (C:N) of the final product (Tiquia & Tam, 2002). Furthermore, high C:N enhances nitrogen uptake efficiency and reduces nitrogen leaching, thereby improving nutrient retention and environmental sustainability (Holub et al., 2020). Through compost, nutrient restoration to the soil can be achieved by reducing the formation and release of carbon- and nitrogen-based volatile compounds into the atmosphere, thereby reducing total GHG emissions.

Nevertheless, nutrient leaching and detrimental gas emissions, including GHGs, remain major concerns associated with the composting process, as deviation of raw material properties, including balanced C:N, oxygen supply, and pH, could lead to undermine quality and reduce agroeconomic aspects (Weindorf et al., 2011).

As an alternative measure, biochar production through the pyrolytic conversion of PL provides waste reduction and long-term carbon sequestration benefits. Biochar, as a stable carbonaceous compound, has a high ability to adsorb organic carbon and prevent oxidation to form volatile CO_2. During pyrolysis, nitrogen- and oxygen-containing compounds in PL are rapidly decomposed, resulting in the formation of a highly stable carbonaceous structure (Yang et al., 2024) with a lower degradation rate compared to untreated PL.

The natural porosity structure of biochar acts as a natural habitat for microbes, hyphae, flora and fauna, with other advantages such as improving soil aeration, water retention, and binders for heavy metals and nutrients, efficiently immobilising pollutants and improving crop productivity (Biswal & Balasubramanian, 2025). Furthermore, it promotes the growth and activity of methanotrophic bacteria, which enhance CH_4 oxidation, while also providing an adsorption platform for inorganic nitrogen, thereby reducing the production of N_2O (Amirahmadi et al., 2025). In addition, lower CO_2 emissions have been observed when organic waste is co-applied with biochar, as biochar has the ability to reduce the carbon degradation rate and enhance carbon stabilisation (Palansooriya et al., 2025), acting as a powerful carbon sink strategy. On top of these characteristics, biochar therefore represents a comprehensive and sustainable waste management pathway that integrates climate benefits with farming enhancement.

Despite these benefits, potential environmental concerns may arise during biochar production, particularly due to the emission of smoke and syngas generated during the pyrolysis process. Therefore, a trade-off assessment of greenhouse gas (GHG) emissions should be conducted in future studies to determine the net GHG impact of each mitigation approach.

Interestingly, the overall difference in total carbon mitigation between composting and biochar conversion was minimal, approximately 5%, corresponding to less than 0.1 kg CO_2-eq/kg LW. This indicates that both methods offer comparable emission reductions within the current LCA boundary. To improve the accuracy of these estimates, future studies should consider expanding the LCA system boundaries to include energy consumption, process emissions, and transportation activities associated with each treatment method. Such comprehensive review would allow a more accurate assessment of net GHG mitigation potential and identify the true environmental trade-offs among different PL management strategies.

Total Solids and Volatile Solids

Table 7 represents the weekly TS and VS content in PL, from day-old chicks to market age. Both TS and VS reached their peak concentrations at week 3 of the birds' age, scoring 63.05% and 97.51%, respectively. The elevation of volatile compounds in excreta could be explained by dietary adaptation, shifting from starter to grower feed, which affected the gut microbial activities and nutrient absorptions, and consequently affected the excreta compositions. Despite this, TS concentrations did not vary significantly across different ages ($p = 0.30$), whereas VS concentrations showed statistically significant differences throughout the production cycle ($p < 0.05$).

Table 7
Weekly total solids (TS) and volatile solids (VS) mean values of the poultry litter (PL)

Composition	Mean	P-value	*Przybulinski et al. (2023)*	P-value	*Marchioro et al. (2018)*	P-value
TS (%)	61.41		62.91	0.40	76.0	0.15
Week 1	60.13	0.30				
Week 3	63.05					
Week 5	61.05					
VS (% of TS)	94.62		91.36	<0.05	64.0	<0.001
Week 1	95.28[a]	<0.05				
Week 3	97.51[a]					
Week 5	91.08[b]					

Note. Means within a column followed by different letters (a-b) are significantly different (P<0.05)

However, when compared to other findings presented by Przybulinski et al. (2023) and Marchioro et al. (2018), VS values assessed from this study showed significant higher amount, while TS showed insignificant differences. High VS concentrations may be attributed to a higher proportion of biodegradable organic matter, such as wood shavings, in the manure. This composition likely contributed to increased VS emissions during the degradation process (Li et al., 2019).

In order to minimise the effects during waste treatment, TS and VS values are crucial indicators (Peces et al., 2014). A higher VS content indicates a greater proportion of biodegradable organic matter, which enhances the biological treatment potential and contributes to effective waste minimisation. High VS content is often associated with higher GHG emissions potential, as the abundant biodegradable organic matter can be readily decomposed and converted into various gaseous products under both aerobic and anaerobic conditions. Nevertheless, the findings of the current study demonstrate a higher proportion of degradable compounds compared to those reported in previous studies.

Table 8
Total solids (TS) and volatile solids (VS) mean values of the poultry litter (PL) [wood shavings-based PL] when comparing with Collins et al. (2023) [straw-based PL], Wedwitschka et al. (2020) [mixture of chicken manure from different sources], and Mohd Johari et al. (2020) [fresh poultry manure]

Composition	TS (%)	P-value	VS (% of TS)	P-value
Mean	61.41		94.62	
Collins et al. (2023)	84.9	<0.001	84.3	<0.001
Wedwitschka et al. (2020)	78.3	<0.001	69.5	<0.001
Mohd Johari et al. (2020)	23.79	<0.001	77.0	<0.001

For better evaluation of different housing systems on the GHG production potential, straw-based PL (Collins et al., 2023), mixtures of chicken manure with other sources (Wedwitschka et al., 2020), and fresh poultry manure (Mohd Johari et al., 2020) production systems were summarised in Table 8. The results revealed highly significant differences in both TS and VS values relative to those obtained in this study.

Fresh poultry manure exhibited the lowest TS value (23.79%), followed by chicken manure from different sources (78.3%). Low TS content was attributed to high moisture content, which could range from 53-78% (Khodadadi et al., 2024), leaving a low proportion of dried residues and organic materials after treatment. Thus, resulting in decreased TS concentration.

Based on these results, the water-holding capacity of different bedding materials can be ranked as follows: straw-based PL > mixture of poultry manure with other sources > wood shavings PL > fresh poultry manure. Furthermore, the total moisture content is influenced by storage method and duration, with a significant decrease observed during the first two days of storage, and after 28 days, particularly under freeze-drying conditions (Pan et al., 2009).

In the setup of Wedwitschka et al. (2020), chicken manures were mixed from various sources, including bedding materials, storage periods, and housing methods. Although these mixtures were expected to contain higher levels of biodegradable organic matter, resulting in higher VS, the results instead showed the lowest VS values compared to individual housing systems (69.5% on a TS basis), suggesting low-degradable organic matter compositions compared to fresh poultry manure and PL. Therefore, the mixture demonstrated the lowest potential for VS production, which is a key parameter in evaluating biogas potential and energy recovery through anaerobic digestion (Wang et al., 2023).

Total solids (TS) represent the total dry matter content, consisting of both degradable and non-degradable components. According to Le Riche et al. (2017), a high TS content may promote crust formation, which can alter aerobic and anaerobic conditions that influence GHG production, including CO_2, N_2O, and CH_4. Furthermore, high VS content indicates the presence of greater amounts of biodegradable organic matter, including nitrogen-containing

compounds, which can be transformed into GHGs through microbial processes. Therefore, lower TS and VS contents are generally preferred in mitigation approaches to reduce the potential formation and emission of GHGs.

Based on these findings, the potential of various feedstock substrates for biogas or power generation can be ranked as follows: wood shavings-based PL > straw-based PL > fresh poultry manure > mixture of poultry manure with other sources. This ranking reflects the availability of biodegradable organic matter represented by VS content. For waste management strategies aiming to minimise GHG emissions, the preferred sequence should follow the same descending order, as substrates with higher biogas potential allow greater conversion of organic matter into energy while reducing the amount of degradable material remaining for uncontrolled decomposition and subsequent GHG emissions.

Nevertheless, statistical comparisons of significant differences among these studies could not be conducted due to insufficient primary data.

IMPLICATIONS OF THE STUDY

The study acts as a milestone for the poultry industry in Malaysia to develop nation-based data, reducing full dependency on the global default value, which is often reported to have some deviations in the accuracy of local results.

In addition, the results of the data allow the broiler company to achieve an Environmental, Social, and Governance (ESG) certificate, improving its competitiveness and sustainable footprint.

LIMITATION AND RECOMMENDATION FOR FUTURE RESEARCH

As the study focuses on the direct and upstream emissions, impacts produced from land-use change and manure management were excluded. Carbon emissions produced during manure management such as anaerobic digestion and composting should be evaluated in future studies to minimise environmental impacts. A more comprehensive LCA, quantifying the net carbon footprint, should be done, including the emissions during biochar production to review the effectiveness in carbon reduction.

Another potential mitigation approach is through feed modification, evident from this study in highest influence in GHG emissions throughout the production. For instance, reduction of N content in PL could significantly reduce N_2O formation. Total methanogens in gastrointestinal tracts or excreta could presumably be quantified as one of the indicators in assessing emissions potential. Compared to the high installation cost for technologies, nutrition modifications act as a more financially friendly strategy and could potentially be implemented worldwide.

Moreover, housing systems could also influence carbon emissions. Implementations such as slatted floors, cages, or free-range production vary in terms of manure management,

exposure, and flock performance, which lead to different emission profiles. Assessing these alternative systems is recommended to guide environmentally sustainable housing practices.

In upcoming research, total volatile fatty acids (VFAs), including butyric and acetic acids, could be integrated into poultry manure analysis, due to their high volatile properties and CH_4 production, which negatively impact the environment (Yin et al., 2022). Also, it could serve as an indicator of microbial activities, assist in minimising GHG emissions, and prediction for biogas potential.

Lastly, environmental pollution potential and bio-reactant capacity could be assessed by quantifying N, CO_2, and CH_4 concentrations in poultry manure, with varying mixtures and moisture contents. These parameters not only reflect the potential for anaerobic digestion and biogas production but also provide insight into possible local environmental pollution during storage (Higgins et al., 2021).

CONCLUSION

In summary, the conventional broiler production system assessed in this study generated a cumulative total of 63.97 tCO_2-eq/house per production cycle. The largest share of emissions (39%) originated from feed procurement (excluding LUC), followed by energy consumption (37%) and manure management (24%). Scope 3 emissions accounted for the highest proportion of the carbon footprint, followed by on-farm emissions under Scope 1. In the absence of mitigation measures, total emissions per house per cycle were estimated at 1.54 kg CO_2-eq/ kg LW of bird. Although the sale of PL offers the highest potential emission reduction (-29.87%), its associated negative environmental impacts make it a less favourable option. Alternatively, converting PL into compost or biochar presents a more sustainable mitigation strategy, reducing total emissions to 1.30 kg CO_2-eq/ kg LW and 1.23 kg CO_2-eq/ kg LW, respectively.

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Review Article

Productivity of Napier grass (*Pennisetum purpureum*) in Response to Nitrogen Fertiliser Application in Tropical Regions: A Meta-Analysis

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ABSTRACT

The major forage in tropical livestock systems is napier grass (Pennisetum purpureum), and the first input applied to improve its yield is nitrogen. How big a response that is remains to be seen. Field trials are discordant, and many compare multiple nitrogen rates to one untreated control, giving undue weight to the evidence. This meta-analysis synthesises the tropical record that accounts for that dependence. English-language articles were retrieved from Scopus, ScienceDirect and Google Scholar following PRISMA. Effect sizes are reported as Hedges' g and pooled using a random-effects model with cluster-robust variance estimation, with each study (rather than each comparison) serving as the unit of analysis. Twelve studies fulfilled the criteria. Nitrogen increased organic matter, crude protein, plant height, fresh biomass yield and tiller number ($P < 0.05$), crude protein being the most consistent response. There were no changes in dry matter, crude fibre, NDF or ADF. The ether extract, stem diameter, leaf area and leaf number were only studied twice each and could therefore not be reliably assessed. There is no single optimal rate that can be defended because few dose subgroups included 3 independent studies. The practical message is not as broad as the primary literature suggests. Moderate nitrogen supply, before the stand lignifies, increases protein and tillering, but higher rates add little.

Keywords: Chemical composition, forage yield, meta-analysis, napier grass, nitrogen fertilisation

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INTRODUCTION

Forage carries most of a ruminant's diet, commonly 60 to 80% of intake, and it is

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the cheaper half of the ration. In the tropics, that supply has to run year-round, yet the swing between wet and dry seasons keeps interrupting it (Loresco et al., 2019; Monte & Marcos, 2024; Umami et al., 2015). Grasses fill much of the gap, since they yield heavily and tolerate marginal conditions. Forage is also a large share of production cost, so the way a stand is managed sets its value. Quality decides what each animal gains; yield decides what a hectare returns.

Napier grass (*Pennisetum purpureum* Schumach.), also known as elephant grass, is one of the principal forage species of the tropics. Native to sub-Saharan Africa (Muktar et al., 2023), it has since been cultivated across tropical and subtropical regions. The species is a tall, tufted perennial characterised by rapid regrowth and high biomass potential, and it remains productive on soils of low fertility and under limited water (Islam et al., 2023; Rusdy, 2016). Rusdy (2016) reported it to be the highest-yielding of the tropical grasses, which accounts for its use by smallholders and commercial producers alike, as a cut-and-carry forage and as a silage crop to maintain feed supply through the dry season (Kaewpila et al., 2020).

The performance of a livestock system rests on both the yield and the quality of its forage, and soil fertility, managed chiefly through fertilisation, is the principal means of improving them (Umami et al., 2015). Maleko et al. (2019) reported that balanced fertilisation reliably promotes forage growth. Under warm climates, the nutritional composition of the plant, including its energy, protein, and mineral content, is sensitive to management; where these attributes are suboptimal in a grass such as Napier, animal performance declines accordingly (Mutimura et al., 2015).

Getting the agronomy right, especially the fertiliser rate and timing of the cut, is key to a heavy crop. Nitrogen is the first nutrient to run short in the bulk that a stand takes out (Knoll et al., 2013; Rengsirikul et al., 2011). It is highly sensitive to Napier grass. As the rate increases, yield also increases up to a ceiling, since nitrogen builds the chlorophyll and the new leaves that drive photosynthesis (Oliveira et al., 2015; Zewdu et al., 2002). However, evidence is limited, and few studies have identified the actual role of nitrogen in regulating growth and quality of this grass.

Nitrogen has been central to yield and nutritive value, and this has increased interest in forage quality and in production efficiency. It's not just the rate that matters. Plant composition and vegetative growth are also influenced by the source and form of nitrogen. A sustainable system must balance the inputs and the storage and uptake of the plant, as over-accumulation degrades quality (Kering et al., 2012; Monti et al., 2008) and increases cost (Aravindhakshan et al., 2011; Haque et al., 2009). Reported responses in Napier grass are inconsistent and vary with dose, environment and management. This meta-analysis was designed to quantify, in tropical conditions, the global effect of nitrogen on the growth, yield and nutritional quality of the grass.

MATERIAL AND METHODS

Literature Search and Study Selection

The review was conducted in the light of the PRISMA framework (Selcuk, 2019). The objective was to collect published information on the influence of nitrogen fertilisation on the nutrient composition and morphology of Napier grass (Pennisetum purpureum) in tropical environments. Three sources were searched, namely, Scopus (https://www.scopus.com), ScienceDirect (https://www.sciencedirect.com), and Google Scholar (https://scholar.google.com). Keywords: "Nitrogen Fertiliser", "Pennisetum purpureum", "Nutrient Content" and "Morphology".

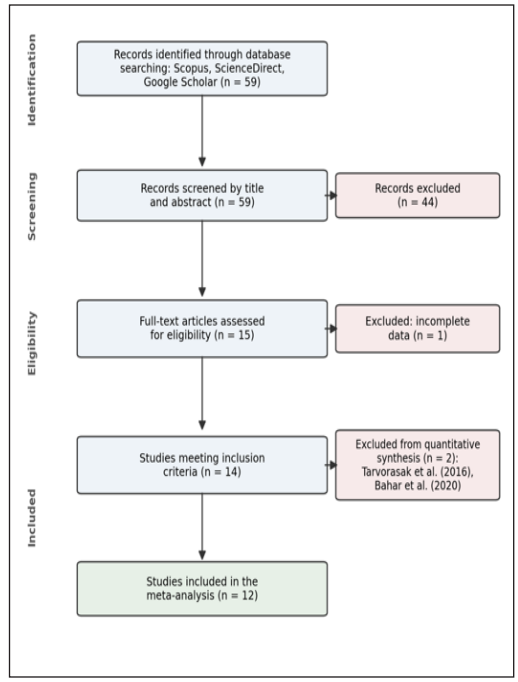


Figure 1. PRISMA flow diagram of the literature search and study selection process

All retrieved articles were screened using a fixed set of inclusion criteria. Inclusion criteria for a study were: (1) peer-reviewed publications in English; (2) experimental work on Pennisetum purpureum of any cultivar or variety; (3) carrying nitrogen fertilisation treatments with matching controls; (4) reporting quantitative data on nutrient composition, morphological traits or both; and (5) reporting details of replication and experimental design. Out of the initial screening, 59 articles were relevant to nitrogen effects on Napier grass in the tropics. A close look at titles and abstracts reduced that to 15. One more was then dropped for incomplete data, leaving 14 that met the criteria; after the two exclusions set out below, 12 studies remained in the quantitative synthesis (Table 1). From these we pulled yield, dry matter, organic matter, crude protein, ether extract, crude fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF), tiller number, stem diameter, plant height, leaf area, and leaf number. Figure 1 lays out the selection process, which followed the PRISMA protocol for systematic reviews and meta-analyses.

This meta-analysis covered the Napier grass complex, defined here as *Pennisetum purpureum* Schumach. (syn. *Cenchrus purpureus*) together with its interspecific hybrids with pearl millet (*P. glaucum*, syn. *P. americanum*). Both pure cultivars (for example, Mott, Taiwan, Red, and BRS Capiacu) and interspecific hybrids (Napier Pakchong 1 and King grass) were included, because the hybrids are derived from *P. purpureum*, are grown and harvested as cut-and-carry forage under comparable management, and are reported in the literature as hybrid Napier grass (Dokbua et al., 2021; Tarvorasak et al., 2016; Muktar

et al., 2023). Variety type, pure cultivar or interspecific hybrid, was recorded for every observation and carried into the subgroup analysis as a moderator.

Indexing was deliberately left out of the inclusion rules. Pairing Scopus and ScienceDirect with Google Scholar reaches a wide range of national and regional journals, so a peer-reviewed study in a national outlet still qualified as long as it met the data and design criteria above. If we had only considered the Scopus-indexed pool, most of the tropical Napier and Pakchong work would have been removed as it is more likely to be published in national journals. We did maintain the English language criterion, for the sake of uniformity in data extraction, and accept that this may leave local studies in other languages under-represented.

Table 1
Studies included in the meta-analysis of nitrogen fertilisation in Napier grass

Reference	Country	Variety (Type)	N Source	N rate, kg/ha (basis)	Harvest Age (d)
Veraque & Gorne (2023)	Philippines	Napier (pure)	Urea	0, 30, 60, 120, 240 (per application)	60, 105
Phakamas & Yampracha (2018)	Thailand	Pakchong 1 (hybrid)	Urea	0, 62.5 (soil-test based)	75
Dokbua et al. (2021)	Thailand	Hybrid Napier (hybrid)	Ammonium sulfate	0, 250, 500, 1000 (seasonal total)	70 (6 cuts)
Rukmana et al. (2024)	Indonesia	Red Napier (pure)	Urea	0, 100, 200, 300 (per application)	70
Syam et al. (2021)	Indonesia	Taiwan (pure)	Urea	0, 300 (per application)	60
Widodo et al. (2019)	Indonesia	Mott (pure)	Urea	0, 150, 300 (per application)	42
Fauzi et al. (2019)	Indonesia	Mott (pure)	Urea	0, 300 (per application)	60
Fauzi et al. (2020)	Indonesia	Taiwan, Mott (pure)	Urea + ammonium sulphate	0, 500, 1000 (per application)	60
Alves et al. (2022)	Brazil	BRS Capiaçu (pure)	Protected urea	0, 100, 200 (kg N/ha/year)	60, 90, 120
Hendarto & Setyaningrum (2022)	Indonesia	King grass (hybrid)	Manure & NPK/urea	50, 75, 100 (per application)	60
Zewdu et al. (2002)	Ethiopia	Napier (pure)	Inorganic N (+ manure arm)	0, 46, 92 (per cut)	60
Girgiri et al. (2024)	Nigeria	Napier (pure)	Poultry manure (organic)	0, 42, 56 (N-equivalent)	84

Data Extraction

According to Higgins and Deeks (2008), a meta-analysis systematically applies statistical methods to synthesise and quantify effect sizes derived from multiple studies. The effect size represents the magnitude and direction of a treatment's impact based on quantitative observations of a specific phenomenon. In this study, all variables meeting the inclusion criteria were extracted from the selected articles, including the mean values and measures of variance (standard deviation or standard error) for the outcome variables of interest in both treatment and control groups. Standard error (SE) values were converted to standard deviation (SD) following the method described by Higgins and Deeks (2008), using the formula $SD = SE \times \sqrt{n}$, where n denotes the number of samples in each treatment group. As the studies applied varying nitrogen fertiliser rates, all fertilisation doses were standardised and expressed in kilograms per hectare (kg/ha). The nitrogen application rates were then categorised into four levels: A = 0-100 kg/ha, B = 100-250 kg/ha, C = 250-500 kg/ha, and D > 500 kg/ha. All extracted data from the included studies were compiled and organised in Microsoft Excel 2021 in comma-separated value (CSV) format, which is compatible with subsequent statistical analyses.

Statistical Analysis

Statistical analyses were performed using the OpenMEE software (Wallace et al., 2017) to calculate the standardised mean differences (SMD) between nitrogen fertiliser treatments and the control group. Nitrogen fertiliser dose was treated as a fixed effect, while variation among studies was treated as a random effect. The comparisons were based on SMD values with 95% confidence intervals (CI). Subgroup analyses were also conducted by nitrogen dose category and harvest age to identify potential sources of heterogeneity.

Many of the included trials reported two or more nitrogen rates against one shared control. Their comparisons are therefore not independent. Pooling them as if they were would shrink the variance and inflate the apparent body of evidence, the precise problem flagged at review. To avoid it, the standardised mean differences were combined under a random-effects model with a cluster-robust variance estimator (Hedges et al., 2010; Tipton, 2015), with the study, not the individual comparison, as the unit of analysis. Each parameter is reported with both counts: k comparisons drawn from m independent studies. An estimate was treated as reliable only where m reached three. Heterogeneity was summarised with the I-squared statistic (Higgins et al., 2003), and subgroups by harvest age and nitrogen dose followed the same three-study rule. Two trials were set aside. Tarvorasak et al. (2016) grew the grass in solution culture, where nitrogen is a concentration rather than a field rate, and Bahar et al. (2020) dosed per clump rather than per hectare. One further trial, Dokbua et al. (2021), reported no measure of dispersion and was dropped from the plant-height and stem-diameter analyses, while the acid detergent fibre values of Alves et al. (2022) were rebased to a whole-plant dry-matter basis.

The effect size was estimated as the difference between the experimental and control group means, divided by the pooled standard deviation of the selected treatment group. Heterogeneity among studies was assessed using DerSimonian and Laird's Q statistic (*Chi-square test*) and the inconsistency index (I^2) as described by (Higgins et al., 2003). When *P*-values were less than 0.05, the effect of nitrogen fertilisation was considered statistically significant. A significant result ($P < 0.05$) indicated that different nitrogen fertiliser doses had a measurable effect on the nutrient composition and morphological characteristics of Napier grass grown under tropical conditions compared to the control group.

Each standardised mean difference was calculated within a study, comparing the fertilised treatments with the unfertilised control grown on the same soil. Between-study differences in baseline soil nitrogen therefore do not bias the estimated effect of nitrogen fertilisation, although they may influence the magnitude of the response and contribute to the between-study heterogeneity observed for several parameters. Baseline soil nitrogen was not reported consistently across the included studies, so it could not be included as a moderator, and we acknowledge this as a limitation.

RESULTS AND DISCUSSION

Effect of Nitrogen Fertilisation on Yield

The effects of nitrogen fertilisation on yield and chemical composition are summarised in Table 2. Pooled across the data, nitrogen lifted fresh biomass yield (SMD = 1.19; 95% CI = 0.36 to 2.02; p = 0.020; k = 16 comparisons from 4 studies). The direction is positive, and the effect is significant relative to unfertilized controls. However, only the 60-day harvest age subgroup survived the three-study rule and remained significant (SMD = 1.36, 95% CI = 0.19 to 2.53, p = 0.038). Each dose category was based on 2 studies or fewer and therefore could not be estimated. This leaves any claim about yield per dose on weak ground.

That growth is due to what happens with nitrogen in the plant. It is present in both chlorophyll and protein, so it is responsible for photosynthesis, cell division and growth all at once. A good supply of it will speed up both vegetative growth and biomass accumulation. Liman et al. (2018) reported that appropriate rates of inorganic nitrogen enhance nutrient availability and root uptake, and thus fresh yield. Tropical soils are generally moist, which enhances diffusion and nitrogen uptake (Hendarto & Setyaningrum, 2022; Ismail, 2012), and plant height, tiller number and leaf area increase with this.

The harvest age pulls on nitrogen use as hard as the supply does (Fauzi et al., 2020; Girgiri et al., 2024). Nitrogen fuels young tissue, and biomass builds fast at the start. As the stand matures, lignification and structural growth tend to slow further gain. A young plant has more leaf to stem and more moisture, so it returns more fresh forage; an older one lays down structural mass slowly. Nitrogen also provides the biochemistry necessary to define quality (Leghari et al., 2016). None of this is a straight-line scale. Beyond a

Table 2
Effect of nitrogen fertilisation on yield and chemical composition of Napier grass

Variables	Covariates	N	Studies	SMD	CI 95% (Lower)	CI 95% (Upper)	SE	P-value	I^2 (%)
Yield	Overall	16	4	1.19	0.36	2.02	0.26	0.020	32
	Harvest age								
	60 d	14	3	1.36	0.19	2.53	0.27	0.038	31
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	2	1	—	—	—	—	not estimable	—
	250- 500 kg/ha	2	1	—	—	—	—	not estimable	—
	>500 kg/ha	8	1	—	—	—	—	not estimable	—
Dry Matter	Overall	24	9	0.43	-0.34	1.20	0.33	0.231	47
	Harvest age								
	42 d	2	1	—	—	—	—	not estimable	—
	60 d	17	6	0.52	-0.26	1.30	0.30	0.147	29
	70 d	3	1	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	5	3	-0.33	-1.74	1.07	0.33	0.415	0
	100- 250 kg/ha	6	4	0.06	-4.75	4.87	1.51	0.971	77
	250- 500 kg/ha	5	4	0.30	-1.54	2.14	0.58	0.636	64
	>500 kg/ha	8	1	—	—	—	—	not estimable	—
Organic Matter	Overall	14	4	1.20	0.27	2.13	0.29	0.026	0
	Harvest age								
	42 d	2	1	—	—	—	—	not estimable	—
	60 d	12	3	1.14	-0.24	2.53	0.32	0.071	0
	Range dose								
	100- 250 kg/ha	3	2	—	—	—	—	not estimable	—
	250- 500 kg/ha	3	2	—	—	—	—	not estimable	—
	>500 kg/ha	8	1	—	—	—	—	not estimable	—

Table 2 *(continued)*

Variables	Covariates	N	Studies	SMD	CI 95% (Lower)	CI 95% (Upper)	SE	P-value	I^2 (%)
Crude Fibre	Overall	10	4	-0.31	-2.85	2.23	0.80	0.724	59
	Harvest age								
	42 d	2	1	—	—	—	—	not estimable	—
	60 d	6	2	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	3	2	—	—	—	—	not estimable	—
	250- 500 kg/ha	3	2	—	—	—	—	not estimable	—
Ether Extract	Overall	6	2	-1.67	-9.85	6.51	0.64	0.235	48
	Harvest age								
	60 d	4	1	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	2	1	—	—	—	—	not estimable	—
Crude Protein	Overall	23	8	1.32	0.74	1.91	0.25	0.001	12
	Harvest age								
	42 d	2	1	—	—	—	—	not estimable	—
	60 d	19	6	1.39	0.79	1.99	0.23	0.002	0
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	6	3	1.06	-1.89	4.01	0.69	0.261	42
	100- 250 kg/ha	5	3	2.28	-2.75	7.31	1.17	0.191	52
	250- 500 kg/ha	4	3	1.82	1.26	2.39	0.13	0.005	0
	>500 kg/ha	8	1	—	—	—	—	not estimable	—

Table 2 *(continued)*

Variables	Covariates	N	Studies	SMD	CI 95% (Lower)	CI 95% (Upper)	SE	P-value	I^2 (%)
NDF	Overall	13	4	0.08	-0.68	0.84	0.24	0.756	17
	Harvest age								
	60 d	11	3	0.01	-1.02	1.04	0.24	0.966	27
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	1	1	—	—	—	—	not estimable	—
	>500 kg/ha	8	1	—	—	—	—	not estimable	—
ADF	Overall	6	3	-0.10	-0.69	0.50	0.14	0.554	0
	Harvest age								
	60 d	4	2	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	2	1	—	—	—	—	not estimable	—

Note. N = number of comparisons; Studies = number of independent studies (clusters). SMD = standardised mean difference (Hedges' g) from a random-effects model with cluster-robust variance estimation; SE = standard error; I^2 = heterogeneity. Subgroups based on fewer than three independent studies are reported as not estimable. Shaded P-values are significant at $P < 0.05$

certain point, uptake efficiency declines as the root zone becomes saturated and excess is lost by leaching and volatilisation. Yield is a balance against soil condition and cost to find a workable dose.

Effect of Nitrogen Fertilisation on Chemical Composition

Nitrogen had no effect on dry matter (SMD = 0.43; 95% CI = -0.34 to 1.20; p = 0.231; k = 24 from 9 studies); the 60-day subgroup was no different (SMD = 0.52; p = 0.147) and the three dose bands that could be estimated, 0 to 100, 100 to 250 and 250 to 500 kg N/ha, told the same flat story. Organic matter was an exception. It climbed under nitrogen (SMD = 1.20; 95% CI = 0.27 to 2.13; p = 0.026; k = 14 from 4 studies), although its one estimable subgroup, the 60-day cut, was marginal and non-significant (SMD = 1.14; 95% CI = -0.24 to 2.53; p = 0.071).

Napier grass can store large quantities of dry matter, ranging from approximately 85.4 t/ha with no fertiliser to as high as 130 t/ha with heavy nitrogen (Islam et al., 2023; Tulu et al., 2021). The range is wide because it is influenced by variety, season, site, soil fertility and management. Dry matter is a quality indicator as it contains most of the nutrients (Rengsirikul et al., 2011; Widodo et al., 2019). But the pooled result shows no steady rise in dry matter with nitrogen. Dose and harvest age are more important than the nutrient itself. The clearest response is seen at about 70 days, when the plant is actively growing and is using nitrogen efficiently for new tissue. With the later cuts, the effect is less because of increased lignification and fibre content.

The wide range in dry matter reported by Zetina-Córdoba et al. (2013) among studies is most likely due to the different nitrogen rates applied. The mechanism is well understood: nitrogen availability fuels photosynthesis and nutrient uptake and switches on the growth hormones auxin, cytokinin and gibberellin that divide and enlarge cells and thus build dry matter (Fauzi et al., 2020). At a given rate, the extra nitrogen contributes little, for as the rate increases the efficiency of uptake falls.

Other fractions provided less reliable answers to nitrogen than did organic matter. Most of the treatments showed an increase because the nutrient is used in the synthesis of organic constituents, mainly protein, structural polysaccharides and lignin (Hendarto & Setyaningrum, 2022; Leghari et al., 2016). One exception, however. In the 250-500 kg N/ha range, the response lost statistical significance, indicating that the organic fraction saturates and stops rising when nitrogen is abundant.

This increase in organic matter is linked to the additional fresh and dry biomass produced by faster growth. This fraction is fed by the same nitrogen-fed photosynthesis and hormone activity that splits and swells cells. Cell-wall share fits into that, as a larger structural fraction generally means more dry matter and more organic matter goes along with it. So nitrogen does raise organic matter, but only as much as the rate and the growth stage allow.

Crude fibre was unaffected by nitrogen (SMD = -0.31; 95% CI = -2.85 to 2.23; p = 0.724; k = 10 from 4 studies), and no subgroup of harvest-age or dose met the three-study bar. Ether extract also showed (SMD = -1.67; 95% CI = -9.85 to 6.51; p = 0.235), but this is read with caution because of two studies. Crude protein was the one clear exception, increasing significantly (SMD = 1.32; 95% CI = 0.74 to 1.91; p = 0.001; k = 23 from 8 studies), and the increase persisted both at the 60 day cut (SMD = 1.39; p = 0.002) and in the 250 to 500 kg N/ha band (SMD = 1.82; 95% CI = 1.26 to 2.39; p = 0.005), with the lower doses remaining flat.

Nitrogen affects the chemical fractions unevenly. As a rule, it reduces the crude-fibre content, but seldom to any appreciable extent. This is because nitrogen prefers young, metabolically active tissue. So the structural pieces, cellulose, hemicellulose and lignin, make up a smaller portion of the whole than they would in an unfertilized plant. As Muhtarudin et al. (2020) put it directly, a better supply of soil nutrients sharpens the plant's control over its cell walls and holds the fibre down.

The small, non-significant increase at 250-500 kg/ha suggests a threshold. Further, as the plant matures, its cell walls thicken, and it develops structural fibre. This is in agreement with Kumari et al. (2022), who reported that inorganic fertilisation improves tissue quality, while nutrient deficiency limits wall formation. Crude fibre cuts both ways. Too much reduces digestibility, increases rumen retention time and pulls productivity down; too little is no better, because ruminants need fibre for normal rumen pH and microbial balance (Goldan et al. 2023; Tuturoong et al., 2020). Fahey et al. (2019) and Hendarto and Setyaningrum (2022) said that lower fibre forage is generally preferred and results in better performance as long as there is sufficient fibre. Age of harvest is important, too, as older stands have more structural tissue.

Nitrogen did not affect ether extract (SMD = -1.67; p = 0.235). The figure is based on only two independent studies, so caution is advised. The lower values are consistent with nitrogen forcing the plant to produce new tissue rather than lipid. This is the normal behaviour of a well-fed plant where dividing and expanding cells are the priority. Plant moisture is also at odds with the ether extract: crude fat increases slowly as the plant matures and dries. Young plants are at the other end, full of dividing cells. Where it has been measured head-on, ether extract in tropical grasses follows regrowth age and season to a much greater degree than it follows fertilisation (Acosta-Balcazar et al., 2024).

Similarly, Maleko et al. (2019) reported that high doses of fertilisers can alter ether extract, and Goldan et al. (2023) connected nutrient supply to growth, biomass, and general quality. Here, a fall in ether extract may be due to breakdown of triglycerides into fatty acids and alcohols. Volatile fatty acids evaporate and pull the fraction down. The ether-extract fraction contains glycerol esters, fatty acids, and fat-soluble vitamins, all of which are likely to vaporise. The fatty acids, on the other hand, protect the surface tissue of the plant, but also serve as an energy store.

Crude protein elicited the strongest and most steady response of all. Nitrogen is the building block of amino acids and protein, so adding more of it directly increases protein synthesis. The literature agrees that a better nitrogen supply improves grass yield, nutritive value and animal performance (Delevatti et al., 2019). Widodo et al. (2019) found that grass given nitrogen alone produced more protein than grass under rotational or traditional cropping, a finding echoed in their results. Here, the response was sustained over nearly all harvest ages and most doses, identifying nitrogen as the principal limitation on protein formation in this grass. When the inorganic fertiliser is well composed, a good supply keeps the meristems active, pushes out new leaves and lifts metabolic rate and protein banks up in the biomass while dry matter and yield rise alongside it (Hendarto & Suwarno, 2018).

Tropical grasses are usually about 10% crude protein, and more nitrogen will usually push the numbers up. And that's pretty much what we saw. Nitrogen has been shown to increase both growth and the accumulation of biomass rich in protein. But the gain is capped by maturity: protein decreases as the plant lignifies and its metabolism slows down. Bayble et al. (2007), Tshering and Penjor (2016) and Wangchuk et al. (2015) recorded sharp protein losses at longer cutting intervals. The evidence taken together suggests that nitrogen accelerates protein-rich tissue in advance of structural tissue, which is why crude protein is so reliably higher in fertilised Napier grass.

Neither fibre fraction moved nitrogen. There was no difference in NDF (SMD = 0.08; 95% CI = -0.68-0.84; p = 0.756; k = 13 from 4 studies), and the subgroup of 60 days was also stable. Whole-plant dry-matter basis ADF was also maintained (SMD = -0.10; 95% CI = -0.69 to 0.50; p = 0.554; k = 6 from 3 studies). This ADF figure corrects an earlier extraction that had entered the annual ADF yield in kg/ha, rather than concentration, which inflated the effect. Plant age, proportion of stem and lignification distinguish both fractions much more than nitrogen (Jimoh et al., 2019; Ojo et al., 2013), and the pooled estimates show the same.

Among all the traits, NDF and ADF tracked nitrogen the least. It hardly registered across treatments, which suggests that structural fibre responds more to plant age than to nitrogen supply. At 60 and 84 days, the stands had matured and lignified, and there was little opportunity for nitrogen to rework the cell wall. There were no categories with three studies, leaving patterns at 100 to 250 kg/ha uninterpreted, and overall nitrogen did not shift either fraction (NDF SMD = 0.08, p = 0.756; ADF SMD = -0.10, p = 0.554). The physiology is simple: both NDF and ADF increase with age as stem fraction and lignin accumulate (Jimoh et al., 2019). High levels of both cut digestibility and intake (Lardner et al., 2015; Nasution et al., 2025). Ojo et al. (2013) also found older plants to be richer in both and poorer as feed.

Effect of Nitrogen Fertilisation on Morphological Traits

The effects of nitrogen fertilisation on the morphological traits are summarised in Table 3. Nitrogen increased plant height with a significant effect (SMD = 0.99; 95% CI = 0.09 to 1.90; p = 0.035; k = 21 from 8 studies). It remained at the 60-day cut (SMD = 0.88; 95% CI = 0.21- 1.54; p = 0.021). All three respective dose bands (0-100, 100-250, 250-500 kg N/ha) pointed upwards; none were significant. In contrast, stem diameter did not respond (SMD = 0.40; p = 0.780). It is reported here for description only based on two studies and a very wide interval.

Nitrogen is part of chlorophyll, amino acids and protein, the materials a plant uses to divide and lengthen its cells, so height increases. Give it enough, and division at the shoot apex runs faster, so the plant stands taller than its unfertilized neighbours. Stronger photosynthesis and steady auxin levels further stretch the internodes (Vennila & Sankaran, 2017), and the stretching is further when the other nutrients and nitrogen arrive together (de Oliveira et al., 2020).

Rukmana et al. (2024) related the height to persistent division in the apical meristem, where the nitrogen supply and plant age together elongate the internodes and increase the volume. Budiman et al. (2012) reported that the height and stem ratio of the crop increased from 8 to 12 weeks (Fauzi et al., 2020). Height is highly correlated with nitrogen availability, which is a major constraint of vegetative growth. The gains at most harvest ages show that plants still in the vegetative phase are spending nitrogen efficiently on photosynthesis and new tissue. The flat response at 84 days tells the opposite story, of heavier lignification and a slower metabolism blunting nitrogen's pull on elongation. Altogether, nitrogen promotes stem growth, vegetative development and carbon assimilation, and this is accompanied by greater height (Dokbua et al., 2021).

Height is more sensitive to nitrogen than stem diameter. In early growth, the nitrogen goes into elongation rather than thickening, so the diameter hardly changes. The increase in radius is related to the development of structures, increases with the age of the plant and the multiplication and swelling of its cells (Zailan et al., 2018) and contributes to the increase of height, volume and size in total. A thicker stem carries more, supports more biomass and better transports water and nutrients (Nasution et al., 2025). And because stems have more lignocellulose and fewer nutrients than leaves (Dokbua et al., 2021), what happens to the stem sets the fibre profile of the whole plant.

Most previous work found that the stem diameter was unaffected by nitrogen rate (Stida et al., 2018), although de Oliveira et al. (2015) observed some movement at the higher end. Above 500 kg/ha, diameter may slightly increase as the plant strengthens its structure to support the additional biomass, but this extra structural material comes at the expense of digestibility and quality (Acosta-Balcazar et al., 2024). Nitrogen is first to division and elongation, so height and leaf expansion precede stem thickening and the diameter is much the same as the rate increases.

Table 3
Effect of nitrogen fertilisation on morphological characteristics of Napier grass

Variables	Covariates	N	Studies	SMD	CI 95% (Lower)	CI 95% (Upper)	SE	P-value	I^2 (%)
Plant height	Overall	21	8	0.99	0.09	1.90	0.38	0.035	51
	Harvest age								
	60 d	15	5	0.88	0.21	1.54	0.24	0.021	23
	70 d	3	1	—	—	—	—	not estimable	—
	75 d	1	1	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	4	3	0.66	-5.06	6.38	1.33	0.669	74
	100- 250 kg/ha	5	3	2.63	-6.49	11.74	2.12	0.341	79
	250- 500 kg/ha	4	3	1.63	-1.02	4.28	0.62	0.118	61
	>500 kg/ha	8	1	—	—	—	—	not estimable	—
Stem diameter	Overall	12	2	0.40	-13.67	14.46	1.11	0.780	50
	Harvest age								
	105 d	4	1	—	—	—	—	not estimable	—
	60 d	8	2	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	6	2	—	—	—	—	not estimable	—
	100- 250 kg/ha	6	2	—	—	—	—	not estimable	—
Number of tillers	Overall	27	7	0.83	0.07	1.59	0.31	0.036	31
	Harvest age								
	60 d	17	4	1.10	-0.71	2.91	0.57	0.147	43
	70 d	3	1	—	—	—	—	not estimable	—
	75 d	1	1	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	105 d	4	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	9	4	0.69	-0.98	2.35	0.52	0.279	42
	100- 250 kg/ha	8	3	1.38	-2.27	5.04	0.85	0.246	56
	250- 500 kg/ha	2	2	—	—	—	—	not estimable	—
	>500 kg/ha	8	1	—	—	—	—	not estimable	—

Table 3 *(continued)*

Variables	Covariates	N	Studies	SMD	CI 95% (Lower)	CI 95% (Upper)	SE	P-value	I^2 (%)
Leaf area	Overall	9	2	0.97	-1.13	3.07	0.17	0.107	0
	Harvest age								
	60 d	9	2	—	—	—	—	not estimable	—
	Range dose								
	250- 500 kg/ha	1	1	—	—	—	—	not estimable	—
	>500 kg/ha	8	1	—	—	—	—	not estimable	—
Number of leaves	Overall	3	2	0.66	-18.25	19.57	1.49	0.735	67
	Harvest age								
	60 d	1	1	—	—	—	—	not estimable	—
	84 d	2	1	—	—	—	—	not estimable	—
	Range dose								
	0- 100 kg/ha	2	1	—	—	—	—	not estimable	—
	250- 500 kg/ha	1	1	—	—	—	—	not estimable	—

Note. N = number of comparisons; Studies = number of independent studies (clusters). SMD = standardised mean difference (Hedges' g) from a random-effects model with cluster-robust variance estimation; SE = standard error; I^2 = heterogeneity. Subgroups based on fewer than three independent studies are reported as not estimable. Shaded P-values are significant at $P < 0.05$

Nitrogen significantly increased tiller number (SMD = 0.83; 95% CI = 0.07 to 1.59; p = 0.036; k = 27 from 7 studies); the two estimable dose bands, 0 to 100 and 100 to 250 kg N/ha, were in the positive direction but not significant. There was no significant increase in leaf area (SMD = 0.97; 95% CI = -1.13 to 3.07; p = 0.107) in two studies and no effect at all on leaf number (SMD = 0.66; p = 0.735) in two studies.

The leaf number is the inconsistent one. It is more directed by genotype and stage of development than nitrogen, so more leaves are not always reliably produced by adding nitrogen. At the very beginning, nitrogen is used for division and elongation, not for new leaves. Under nitrogen, Napier grass may produce more leaves, but the response varies between varieties. Nitrogen does promote leaf number and leaf area, and thus vegetative growth (Oliveira et al., 2015), a more complete canopy feeding back into biomass. Nasution et al. (2025) also said that cultivars differ in efficiency of nutrient conversion to morphological traits such as leaf number, which is one reason why leaf response is very inconsistent.

Leaf area increased again based on only two studies but not significantly (SMD = 0.97; p = 0.107). Mechanistically, chlorophyll and protein synthesis link nitrogen to leaf expansion, but two studies failed to confirm a real effect. Bigger leaves capture more light and therefore photosynthesise more, and a richer supply of nitrogen increases the rate of photosynthesis and vegetative growth, which appears as taller plants and a fuller canopy. The leaf: stem ratio also increases, indicating that leaves are more responsive to nitrogen later in development. Ojo et al. (2014) linked higher yield with increased leaf area through better light interception, and Ernawati et al. (2023) linked it with increased productivity.

Tiller number showed a clear and consistent response to nitrogen. It promotes cell division and basal meristem activity and increases apical dominance, enabling more axillary buds to develop as tillers. The strong showing at low to moderate rates says that only a modest supply is needed to set tillering off. The effect fades with age as meristem activity drops and lignification takes over. The fertilisation scheme is a clear determinant of tillering in Napier grass (Gorne & Aradilla, 2020), and nitrogen improves yield, quality and tiller production (Hazary, 2015). Nitrogen increases the synthesis of proteins and chlorophyll and enhances photosynthesis to promote both vegetative growth and height and tillering and branching, thus producing more tillers (Syam et al., 2021).

Sources of Variation Among Studies

These responses are attributed to nitrogen, but the performance of Napier grass depends on other factors simultaneously: cultivar, soil fertility, rainfall and irrigation, and cutting schedule. None of these is evenly distributed across the included trials. The clearest case: nitrogen source. Almost all studies used an inorganic form, with urea being the most used; organic nitrogen appears only in Girgiri et al. (2024) who used poultry manure, and in a

single treatment of Hendarto and Setyaningrum (2022). The pooled estimates thus represent inorganic nitrogen and should not be extrapolated to manure-based systems without additional data. Soil fertility and background soil nitrogen were reported too inconsistently to model. Variation in rainfall, irrigation and harvest age add variation that no analysis here could hold constant. "Harvest age is the biggest thing because it pulls against nitrogen on the quality traits. As the plant matures, fibre goes up, and crude protein goes down. Rate and cutting interval have to be read in conjunction, not one at a time.

Variety was used as a moderator in the analysis, pure Pennisetum purpureum cultivars vs interspecific hybrids. The comparison was only possible for tiller number and plant height. For all other traits, hybrids were represented by at most one study. Where it could be run, the hybrids gave larger point estimates, but the confidence intervals were wide and overlapped those of the pure cultivars. The contrast was based on only two hybrid studies. Therefore, the present data do not allow us to say if hybrids react stronger on nitrogen than pure cultivars. We do not infer anything about the gap, but flag it.

Heterogeneity and Publication Bias

Funnel plots are presented in Figures 2 and 3 and show obvious asymmetry. Such asymmetry can be read as a sign of publication bias, in the sense of the smaller or null results not being published. Bias isn't the only source, though. Experimental conditions, nitrogen levels, cultivar, and environment alone will skew a funnel plot. These tests have little power with only a handful of studies per parameter and must be read with care. The

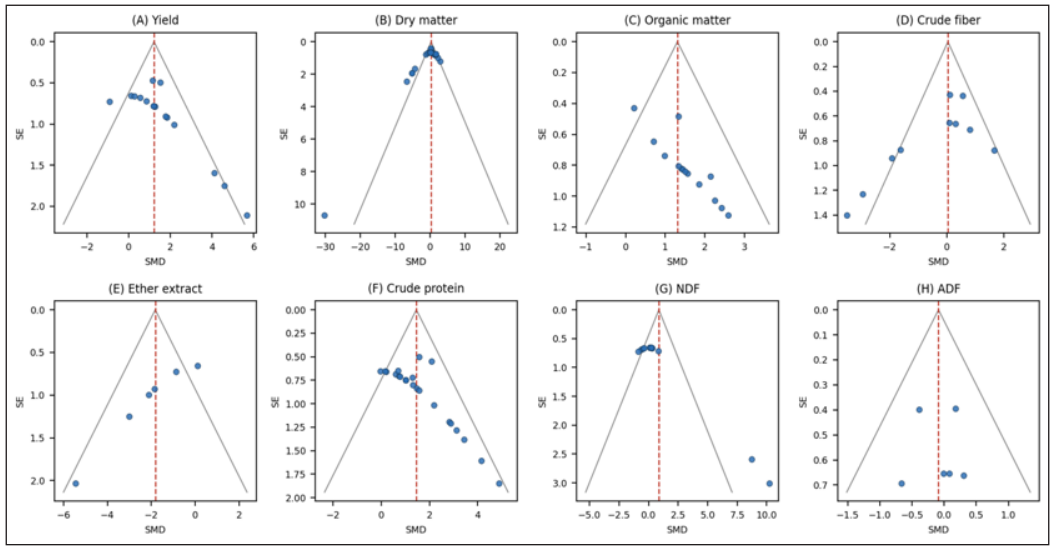


Figure 2. Funnel plot analysis on (A) Yield, (B) Dry Matter, (C) Organic Matter, (D) Crude Fibre, (E) Ether Extract, (F) Crude Protein, (G) Neutral Detergent Fibre, and (H) Acid Detergent Fibre to Detect Publication Bias

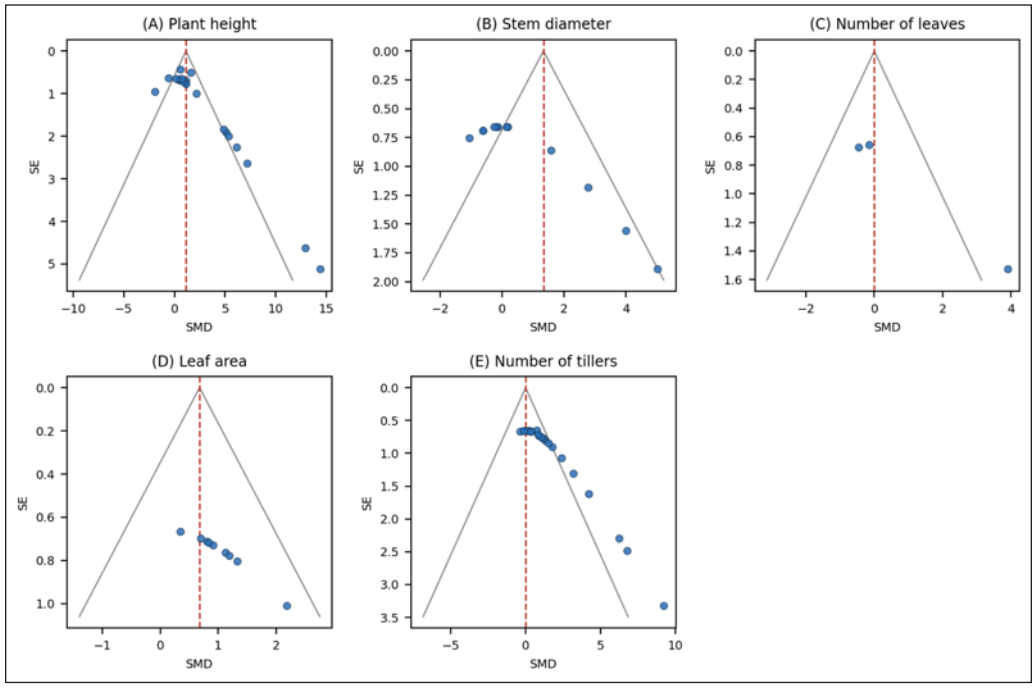


Figure 3. Funnel plot analysis on (A) Plant height, (B) Stem diameter, (C) Number of leaves, (D) Leaf area, and (E) Number of tillers to detect publication bias

broader point is in line with the rest of the analysis: the tropical Napier nitrogen literature is still small and patchy, and its findings are best judged with the number of independent studies underpinning each estimate firmly in mind.

CONCLUSION

However, once the shared-control dependence is dealt with, a clearer picture emerges: nitrogen does more for the nutritive value and vegetative density of Napier grass than for its yield. Crude protein, organic matter and tiller number moved most, each supported by several studies. Fresh yield and plant height behaved similarly. Crude fibre, NDF and ADF remained unchanged, as did dry matter. Four traits, ether extract, stem diameter, leaf area and leaf number, were reduced to two studies each and cannot be concluded. We do not offer any optimal rate. It was never tested head-to-head, and almost every dose subgroup was too thin to estimate. The defensible reading is simpler: moderate nitrogen, applied before the crop lignifies, raises protein and tillering, and pushing the rate higher buys little more. These statements are true for inorganic nitrogen in the cultivars studied here. They do not extend to manure-based systems or to varieties that are untested. The limitation is the evidence base. What the field needs next is straightforward: more independent tropical

field trials that report dispersion for both treatment and control and state the nitrogen rate basis and source outright.

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Agronomic Performance, Forage Yield, Nutritive Value and Forage Utilisation of Four *Urochloa* spp. to Repeated Cutting Cycles in Thailand

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ABSTRACT

Forage crops are fundamental for ruminant sustainable production, but Thailand's crop-livestock systems confront persistent feed shortages and low forage quality. Cultivating high-yield tropical grasses offers a practical solution, but selecting superior species requires detailed agronomic and nutritional evaluation. *Urochloa* grasses represent a promising resource for enhancing feed security. This study evaluated four *Urochloa* varieties (*U. ruziziensis*, *U. brizantha*, *U. decumbens*, and Mulato II) over three cutting cycles to assess growth performance, yield, nutritive value and silage quality under tropical conditions. The field study was conducted from July 2022 to April 2023 in Khon Kaen, Thailand, utilising a randomised complete block design with three replications. Grasses were harvested at 46-50 day regrowth intervals from third cutting, and fresh forage was ensiled in vacuum-sealed HDPE bags for 21 days without additives. In the third cutting, *U. brizantha* produced the highest fresh (16,670.83 kg ha^{-1}) and dry matter yields (4,520.83 kg ha^{-1}). *U. decumbens* showed the highest cell wall fraction, with NDF reaching 61.30% in the first cycle, whereas *U. ruziziensis* and Mulato II exhibited lower fibre contents and greater pre-ensiling digestibility (70.97% and 71.90% IVTDMD, respectively). In silage quality, *U. brizantha* maintained lower NH_3-N levels (0.15%), while *U. ruziziensis* and Mulato II demonstrated high digestibility. Therefore, *U. brizantha* showed exceptional yield potential, while Mulato II and *U. ruziziensis* exhibited desirable nutritional and fermentation characteristics. These findings provide practical guidance for selecting suitable *Urochloa* species for forage production and silage utilisation in tropical livestock production systems.

Keywords: Agronomic performance, forage yield, nutritive value, silage quality, *Urochloa* spp.

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INTRODUCTION

Forage crops are essential as primary sources of roughage for ruminants, particularly beef and dairy cattle, which are key sources of meat and milk (Jayaneththi et al., 2026). In Thailand's crop-livestock mixed farming systems, year-round feed availability is often insufficient. This shortage, combined with poor feed quality, hinders the ability to meet the nutritional requirements of livestock throughout the year (Feyissa et al., 2022; Mottet et al., 2017). The introduction of enhanced forage varieties has been identified as a viable strategy to provide high-quality feed alternatives, thereby improving livestock productivity and addressing feed deficits within such systems (Feyissa et al., 2022). In addition to forage yield, digestibility, silage quality, and climate resilience are important considerations for tropical forage grasses, as these traits influence livestock productivity and adaptation to changing environmental conditions (Adepoju & Faniyi, 2025; Alves et al., 2025; Rodríguez et al., 2024)

The *Urochloa* genus is a tropical forage grasses, which has been cultivated in tropical and subtropical regions as pasture. The genus has about 100 species recognised in the world (Ferreira et al., 2021). At present, some species such as *U. brizantha*, *U. decumbens*, *U. humidicola* and *U. ruziziensis* have been important commercially because these species have many positive attributes, e.g., tolerance to acid soil, high productivity and high-quality forage (Ferreira et al., 2021; Hare, 2023; Masters et al., 2024). *U. ruziziensis* (Ruzigrass), is widely cultivated in Thailand due to its moderate drought tolerance, producing high seed yields, trampling resistance, high nutritional value, and palatability (Department of Livestock Development, 2002). However, limitations such as low seed germination (Usberti & Martins, 2007) and poor waterlogging tolerance (Department of Livestock Development, 2002). Mulato II (*U. ruziziensis x U. brizantha x U. decumbens*), developed by CIAT, is a hybrid forage grass widely cultivated in Thailand. It offers improved seed production and drought tolerance compared to Mulato I (Nakamanee et al., 2007) but remains limited by high seed costs and low waterlogging tolerance (Sithiwong et al., 2010). *U. decumbens* exhibits strong drought and grazing tolerance, while *U. brizantha* outperforms *U. ruziziensis* in drought resistance and dry matter yield (Hare et al., 2005).

However, due to limited seed availability and propagation, *U. brizantha* and *U. decumbens* have not yet been widely cultivated in Thailand. Several studies conducted in the temperate region of Japan by Nitthaisong et al. (2016) investigated the agronomic characteristics, growth, and influence on weeds of six *Urochloa* species. The results showed that *Urochloa* species exhibited stronger growth and higher dry matter yields compared to Rhodesgrass. In Thailand, *U. decumbens* has shown superior adaptability and yield compared to *U. ruziziensis*, producing 41.38% higher yields and 58.59% more during the dry season (Thinnakorn, 1988). It also adapts well to fertile soils, as demonstrated in Khon Kaen province (Wilailpol et al., 1989; Wilailpol et al., 1982). Additionally, *U. brizantha*

outperformed other grasses in dry matter yield (Ernawati et al., 2023; Gatheru et al.,2017; Perini et al., 2023; Ponnampalam et al., 2024).

However, despite their adaptability, previous studies in Thailand have primarily focused on the productivity of individual *Urochloa* species, while comprehensive comparative information on their agronomic performance, nutritional quality, seasonal response to repeated cutting, and silage potential remains limited. This indicated the essential need for comprehensive data on these study species. The study was based on the hypothesis that while top-performing *Urochloa* cultivars sustain high yields across repeated cuttings, their rising fibre content during regrowth poses a challenge for silage quality. Using this data to pinpoint the appropriate harvest time could solve the issue.

The primary significance of this study is that it introduces a multi-harvest selection process for livestock production in tropical conditions. Combining seasonal field performance directly with raw fermentation metrics provides a practical path for year-round sustainability for ruminant production in Thailand.

This study focuses on evaluating four distinct *Urochloa* species for agronomic performance, biomass yield, nutritive value, and the ensiling process of anaerobic fermentation post-harvesting without feed additive supplementation. The results revealed superior forage options suited for tropical conditions, thereby laying the groundwork for more resilient regional production systems.

MATERIALS AND METHODS

The field experiment was conducted at Tha Phra Subdistrict, Mueang District, Khonkaen, Thailand (latitude 16.20° N, longitude 102.49° E, and an elevation of 166.70 m above sea level). The study was carried out from July 2022 to April 2023. The soil type of the field was sandy soil, and rainfall data for the entire experimental period were obtained from the Khon Kaen Meteorological Station, Tha Phra, Mueang, Khon Kaen Province. Four tropical grasses, *U. ruziziensis*, *U. decumbens*, *U. brizantha* and Mulato II were used for comparison.

The field experiment utilized a Randomised Complete Block Design (RCBD) with three replications, while the post-harvest silage experiment followed a Completely Randomised Design (CRD) with four replications. Vegetative roots were transplanted using 2-3 splits per hole at a depth of 10 cm in July 2022 under supplementary irrigation. Each grass was planted in a 6 m^2 plot area (3× 2 m). A basal fertiliser consisting of nitrogen (15% N), double superphosphate (15% P_2O_5) and potassium chloride (15% K_2O) was applied to the plot area at a rate of 62 kg ha^{-1} at transplanting and immediately after each harvest. Weed management and regular monitoring of disease and insects were performed as appropriate.

Agronomic traits (plant height, stem diameter, leaf blade width, and leaf blade length, SPAD and tiller number) were investigated on four randomly selected grasses in each plot

and were measured on the same days of cutting. For yield trait investigation, plants were cut 10 cm above ground level on 16 January, 7 March, and 22 April 2023 at approximately 46-50 days regrowth intervals. Plant materials were separated by hand into leaf blades and stems with leaf sheath to determine the leaf and stem ratio of whole plant dry samples for every yield sample. Plant materials were dried at 60 ºC for 72 hours prior to determining dry weight. Crop growth rate (CGR) was calculated by dividing dry matter yield by the regrowth period (days) between harvests and expressed as kg $m^{-2}d^{-1}$

Nutritive Value

Grass samples were dried at 105 °C overnight and ground to pass through a 1 mm sieve. The total ash content was determined in a furnace at 550 °C for 6 h. Neutral detergent fibre (NDF), Acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al (1991). Crude protein (CP) was determined using the Kjeldahl method. Ash and Ether extract (EE) were determined according to the Association of Official Analytical Chemists (AOAC) (2005).

Silage Quality

The experiment was a completely randomised design (CRD) with four treatments and four replications. Data collection was conducted during day zero (before fermentation) and 21 days after fermentation. Four grass was harvested from the third cutting on 22 April 2023. Then, it was chopped and packed into fermentation bags with HDPE (high-density Polyethylene) type. Air was removed using a vacuum machine, and the bag was sealed with a heat sealer. After that, the silage was stored under ambient warehouse conditions. Fresh forage was ensiled without moisture adjustment or additives. Moisture content was determined before ensiling and after 21 days of fermentation to evaluate changes during the ensiling process. In vitro true dry matter digestibility (IVTDMD) was analysed using an ANKOM Daisy II incubator (ANKOM Technology, 2017). The method has several steps; 0.3 g of samples were prepared in a filter bag (F57), buffer solutions and rumen inoculum mix were prepared, then the bags were incubated in a rumen fluid mix at 39±0.5 ºC for 48 hours. After 48 hours of incubation, bags were washed with the neutral detergent solution in an ANKOM-200 Fibre Analyser to remove rumen fluid particles and non-cell wall material. The bags were dried in a hot air oven, then weighed and recorded as the final weight (W3). The values of samples were used for energy and digestibility estimation. IVTDMD was calculated following ANKOM technology (2017), and *in vitro* Neutral detergent fibre digestibility (NDFD) was calculated following Muttoni et al. (2013).

Statistical analysis

All statistical analyses were carried out with the program R software (version 3.1.1, R Core Team 2021).

RESULTS

Rainfall and Climate

The amount of rainfall was 281 mm in July 2022. The highest amount of rainfall was 455 mm in September (1.5 months after transplanting). The amount of rainfall from July to December in 2022 was 1,136 mm. In 2023, the maximum rainfall occurred in January (188 mm). The total amount of rainfall during the 10-month study period (July 2022 to April 2023) was 1,430 mm (Figure 1). The average temperature was 26.0 - 29.3 °C (Figure 1).

Agronomic Performance

The plant height of four *Urochloa* grasses in the 1st cutting ranged from 30.08 to 48.56 cm and did not show a significant difference. However, significant differences were observed in the 2nd and 3rd cutting, with *U. ruziziensis* (36.47 cm) exhibiting the lowest plant height in the 2nd cutting, while in the 3rd cutting, *U. brizantha* (85.76 cm) exhibited the highest plant height (Table 1). The range of tiller numbers in the 1st cutting was 121.22 to 160.44 and 180.44 to 231.44 tillers per plant in the 2nd cutting. The tiller number showed no significant difference among the varieties in both cuttings. However, there was a significant difference in the number of tillers in the 3rd cutting between *U. ruziziensis* (283.44) and

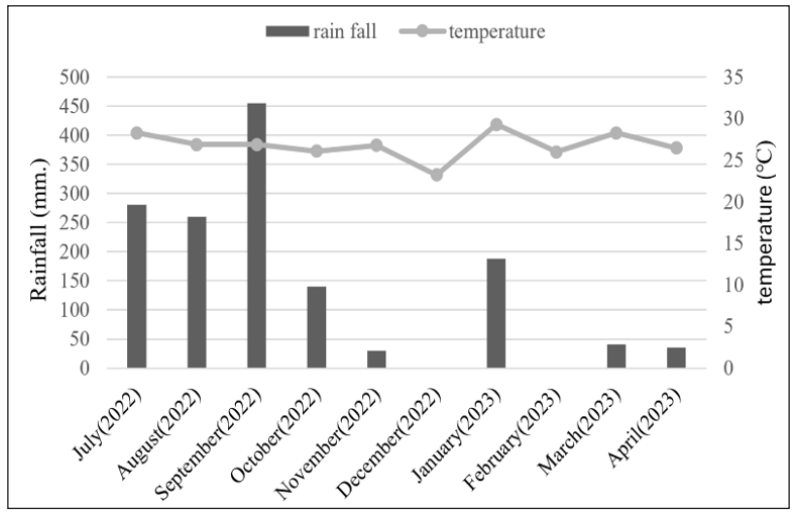


Figure 1. Average monthly rainfall and temperature from July 2022 to April 2023 at the Khon Kaen Meteorological Station, Tha Phra, Mueang, Khon Kaen Province, Thailand

U. decumbens (252.33) exhibiting the highest tiller number compared to Mulato II (Table 1). The stem diameter ranged from 2.58 - 4.33, 1.00 - 2.91 and 2.55 - 3.99 mm in the 1st, 2nd and 3rd cutting, respectively. Significant differences were observed among the varieties in all cutting cycles, with Mulato II and *U. brizantha* exhibiting the largest stem diameter in the 1st and 3rd cutting, and *U. brizantha* showing the highest stem diameter in the 2nd cutting (Table 1). There were significant differences in leaf width and leaf length among the varieties in all cuttings. Mulato II and *U. brizantha* exhibited the highest leaf width in 1stand 3rd cutting while in the 2nd cutting, only Mulato II exhibited the highest leaf width. In terms of leaf length, *U. brizantha* exhibited the highest leaf length in all cutting cycles, and Mulato II also exhibited the highest leaf length in the 2nd cutting (Table 1). The leaf greenness ranged from 35.35- 45.71 and 29.61 - 35.66 SPAD units in the 1st and 3rd cutting. Significant differences were observed among the varieties, with *U. ruziziensis* exhibiting the lowest leaf greenness in the 1st and 3rd cuttings, and *U. decumbens* also exhibited the lowest leaf greenness in the 3rd cutting (Table 1).

Table 1
The growth of U. ruziziensis, Mulato II, U. decumbens and U. brizantha after 1st cutting, 2nd cutting and 3rd cutting

Treatment	Height (cm)	Tiller per Plant	Stem Diameter (mm)	Leaf Width (cm)	Leaf Length (cm)	SPAD
1st cutting						
U. ruziziensis	30.08	160.44	2.62$^{b1/}$	1.64^{ab}	20.50^{b}	35.25^{b}
Mulato II	35.57	121.22	4.33^{a}	1.85^{a}	23.28^{ab}	45.71^{a}
U. decumbens	48.56	128.22	2.58^{b}	1.49^{b}	19.80^{b}	41.60^{a}
U. brizantha	41.50	137.55	3.99^{a}	1.81^{a}	27.78^{a}	45.52^{a}
F-test	ns	ns	*	*	*	*
C.V. (%)	21.32	18.71	19.55	8.24	11.61	6.28
2nd cutting						
U. ruziziensis	36.47^{b}	217.33	1.50^{bc}	1.50^{bc}	25.41^{ab}	nd
Mulato II	47.84^{a}	180.44	1.91^{b}	1.95^{a}	31.65^{a}	nd
U. decumbens	46.07^{a}	189.33	1.00^{c}	1.45^{c}	18.28^{b}	nd
U. brizantha	48.56^{a}	231.44	2.91^{a}	1.88^{ab}	35.51^{a}	nd
F-test	*	ns	*	**	**	-
C.V. (%)	10.43	24.06	14.90	8.02	14.36	-
3rd cutting						
U. ruziziensis	70.82^{bc}	283.44^{a}	2.55^{b}	1.28^{b}	22.01^{b}	29.61^{b}
Mulato II	76.03^{b}	175.33^{b}	3.99^{a}	1.90^{a}	26.53^{ab}	35.66^{a}
U. decumbens	66.18^{c}	252.33^{a}	3.48^{ab}	1.35^{b}	15.99^{c}	29.70^{b}
U. brizantha	85.76^{a}	246.78^{ab}	3.86^{a}	1.80^{a}	30.15^{a}	35.09^{a}
F-test	**	*	*	*	*	*
C.V. (%)	4.23	15.84	16.03	5.04	10.83	5.75

Yield

The yield potential of the four *Urochloa* grasses in the 1st cutting demonstrated significant differences in both fresh and dry weights. *U. brizantha* showed the highest fresh and dry weights with a yield of 4,212.50 and 945.80 kg $ha^{-1,}$ respectively, with no significant difference compared to Mulato II and *U. decumbens*. However, no significant differences were observed in the dry matter contents (DM) among the varieties in the 1st cutting. In the 2nd cutting found that the fresh weight, dry weight, and DM of the four *Urochloa* grasses were significantly different. In terms of fresh weight, Mulato II exhibited the highest value at 7,560.89 kg ha^{-1}, with no significant difference observed compared to *U. decumbens* (6,781.94 kg ha^{-1}) and *U. brizantha* (6,359.65 kg ha^{-1}). For dry weight, *U. decumbens* (1,864.09 kg ha^{-1}) and Mulato II (1,794.21 kg ha^{-1}) gave the highest values, while *U. decumbens* had the highest DM. In the 3rd cutting, significant differences were observed in the fresh and dry weight of the four *Urochloa* grasses. *U. brizantha* exhibited the highest values, with fresh weight reaching 16,670.83 kg ha^{-1} and dry weight 4,520.83 kg ha^{-1}. While DM did not differ significantly among the groups (Table 2), evaluating the relative performance across consecutive cutting cycles underscored a dramatic acceleration in total biomass and structural maturity. Specifically, *U. brizantha* exhibited an exceptional +295.75% relative increase in fresh matter yield and a +377.99% relative increase in dry matter yield from the first to the third cutting cycle, outperforming the recovery velocity of all other varieties. Concurrently, plant maturation led to expected structural cell wall alterations, with *U. decumbens* demonstrating a relative NDF increase of +12.41% by the final harvest. The crop growth rate of the four *Urochloa* grasses, based on the growth rate among all three cutting cycles, indicated no significant difference in CGR1. However, a significant difference was observed in CGR2, which found that *U. brizantha* had the highest CGR value at 10.35 kg m^{-2} day^{-1}, followed by *U. ruziziensis*, *U. decumbens* and Mulato II (Table 3), respectively.

Table 2
Fresh matter yield (kg ha^{-1}), Dry matter yield (kg ha^{-1}) and Dry matter of U. ruziziensis, Mulato ll, U. decumbens and U. brizantha after 1st cutting, 2nd cutting and 3rd cutting

Treatment	Fresh Yield	Dry Yield	DM (%)
1st cutting			
U. ruziziensis	2,520.83$^{b1/}$	564.00^{b}	22.53
Mulato II	3,475.00^{ab}	713.48^{ab}	20.50
U. decumbens	3,716.66^{ab}	782.48^{ab}	21.03
U. brizantha	4,212.50^{a}	945.80^{a}	22.43
F-test	**	**	ns
C.V. (%)	14.60	10.58	6.41

Table 2 *(continued)*

Treatment	Fresh Yield	Dry Yield	DM (%)
2nd cutting			
U. ruziziensis	4,227.58[b]	1,009.01[b]	24.30[cb]
Mulato II	7,560.89[a]	1,794.21[a]	23.76[c]
U. decumbens	6,781.94[ab]	1,864.09[a]	27.47[a]
U. brizantha	6,359.65[ab]	1,607.21[ab]	25.26[b]
F-test	*	*	*
C.V. (%)	23.52	22.09	2.26
3rd cutting			
U. ruziziensis	9,679.16[b1/]	2,700.90[b]	27.91
Mulato II	12,141.67[ab]	3,185.09[b]	26.37
U. decumbens	11,558.33[b]	3,319.35[b]	28.84
U. brizantha	16,670.83[a]	4,520.83[a]	27.11
F-test	**	**	ns
C.V. (%)	12.19	9.60	5.46

Note. ns = non-significant, ** = highly significant ($p \leq 0.01$)
[1/]Means in a column with the same letter are not significantly different based on least significant difference (LSD)

Table 3
Crop growth rate (kg $m^{-2}d^{-1}$) of U. ruziziensis, Mulato II, U. decumbens and U. brizantha after cutting

Treatment	CGR (kg $m^{-2}d^{-1}$)	
	CGR 1	CGR 2
U. ruziziensis	1.58	6.01[b1/]
Mulato II	3.84	4.94[b]
U. decumbens	3.85	5.17[b]
U. brizantha	2.35	10.35[a]
F-test	ns	*
C.V. (%)	40.90	28.24

Note. ns = non-significant, * = significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$)
[1/]Means in a column with the same letter are not significantly different based on least significant difference (LSD)

Nutritive Value

The nutritional analysis in the 1st cutting cycles of all four *Urochloa* grasses revealed that the crude protein concentration ranged from 10.36 to 11.00% (Table 4) and did not differ significantly among the four varieties. However, significant differences were observed in the levels of Ash, EE, NDF, ADF and ADL. *U. decumbens* exhibited the highest levels of NDF, ADF and ADL compared to other varieties. Regarding ether EE concentrations, *U. brizantha* demonstrated the highest values among the evaluated varieties. Meanwhile, *U. ruziziensis*, Mulato II and *U. brizantha* did not have significant differences in Ash level

(Table 4). In the 2nd cutting cycles, the range of CP and Ash levels was 7.40 to 8.40% and 11.00 to 14.26%, respectively, with no significant difference among the varieties (Table 4). In terms of EE, NDF, ADF, and ADL levels, *U. ruziziensis* and Mulato II showed the lowest significant differences in NDF levels when compared to other varieties. Significant differences were observed in the levels of ADF and ADL, with *U. decumbens* exhibiting the highest levels. However, no significant differences were found when compared to *U. ruziziensis* and *U. brizantha* in ADF levels, and only *U. ruziziensis* showed significant differences in ADL levels. At the same time, *U. ruziziensis* and Mulato II exhibited the highest significant EE levels when compared to *U. decumbens* (Table 4). In the 3rd cutting, CP, ADF and ADL were not significantly different among varieties. *U. ruziziensis* had the lowest significant NDF levels when compared to *U. decumbens* and *U. brizantha* (Table 4). Both *U. ruziziensis* and Mulato II showed the highest significant Ash levels when compared

Table 4
The chemical compositions of U. ruziziensis, Mulato II, U. decumbens and U. brizantha after 1st cutting, 2nd cutting and 3rd cutting

Treatment	CP (%)	NDF (%)	ADF (%)	ADL (%)	Ash (%)	EE (%)
1st cutting						
U. ruziziensis	10.73	55.26$^{c1/}$	30.83^{c}	5.13^{b}	16.46^{a}	1.93^{b}
Mulato II	10.36	52.77^{d}	29.50^{c}	4.70^{b}	16.30^{a}	2.03^{ab}
U. decumbens	11.06	61.30^{a}	38.90^{a}	7.03^{a}	13.43^{b}	1.83^{b}
U. brizantha	11.00	58.73^{b}	32.33^{b}	5.60^{b}	14.70^{ab}	2.33^{a}
F-test	ns	*	*	**	*	*
C.V. (%)	6.68	2.00	2.03	6.00	8.29	8.86
2nd cutting						
U. ruziziensis	7.76	56.93^{b}	31.87^{ab}	4.36^{ab}	14.20	2.13^{a}
Mulato II	7.40	57.26^{b}	30.13^{b}	3.57^{b}	14.26	2.03^{a}
U. decumbens	6.86	65.43^{a}	33.97^{a}	4.83^{a}	11.00	1.56^{b}
U. brizantha	8.40	62.90^{a}	32.40^{ab}	3.57^{b}	11.43	1.93^{ab}
F-test	ns	**	*	**	ns	*
C.V. (%)	13.35	3.16	5.08	9.58	12.67	9.64
3rd cutting						
U. ruziziensis	6.86	65.64^{b}	35.14	6.62	9.55^{a}	1.00^{b}
Mulato II	5.60	67.43^{ab}	36.46	6.61	10.93^{a}	0.90^{b}
U. decumbens	5.86	68.91^{a}	35.53	4.93	8.12^{b}	1.16^{a}
U. brizantha	6.03	69.94^{a}	36.51	4.31	8.68^{b}	1.03^{ab}
F-test	ns	**	ns	ns	*	*
C.V. (%)	11.25	1.27	2.83	22.88	9.75	6.70

Note. ns = non-significant, * = significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$)
[1/]Means in a column with the same letter are not significantly different based on least significant difference (LSD)

to *U. decumbens* and *U. brizantha*. *U. decumbens* exhibited the highest significant EE levels, with no significant differences found when compared to *U. brizantha* (Table 4).

Silage Quality

The chemical composition of silage included DM contents and levels of CP, Ash, EE, NDF, ADF and ADL are presented in Table 5. *U. decumbens and U. brizantha* showed the highest significant DM contents compared to other varieties in both before and after fermentation. There were ranges in CP levels among the four varieties of grass before and after fermentation, 7.16 to 9.56 and 7.80 to 10.00% (Table 5), respectively, with no significant difference among the varieties. The range of Ash levels before fermentation was 10.36 to 12.17% and did not have significant differences among the varieties. However, Mulato II had the highest significant Ash concentrations after fermentation (Table 5). The fermentation profiles, including silage pH and NH_3-N concentrations, are shown in Table 5. Significant differences were observed in the silage pH before and after fermentation. Before the fermentation of silage, *U. brizantha* exhibited the highest pH compared to *U. ruziziensis*, whereas after fermentation, *U. decumbens* exhibited the highest pH in comparison to *U. brizantha*. For Ammonia nitrogen (NH_3N) concentrations, *U. brizantha* exhibited the lowest significant NH_3-N concentrations in both before and after fermentation. The digestibility of silage was indicated by IVTDMD and NDFD as shown in Table 5. *U. ruziziensis* and Mulato II exhibited the highest IVTDMD and NDFD compared to other varieties before fermentation, with no significant difference in NDFD observed between *U. brizantha*. However, no significant differences were observed in IVTDMD and NDFD after fermentation.

DISCUSSION

The study evaluated the agronomic performance of four *Urochloa* varieties with three cutting cycles under Thailand conditions, with a particular focus on identifying optimal candidates for sustainable forage production (Dereje et al., 2024). Considering the rainfall prior to each cutting cycle, it was found that a dry spell in February 2023 negatively affected the growth of all four *Urochloa* varieties, resulting in reduced plant height and stem diameter. However, *U. brizantha* continued to demonstrate superior plant height and stem diameter relative to the other varieties, which is attributable to its greater adaptability to environmental conditions (Perini et al., 2023). Enhanced tillering constitutes a key adaptive mechanism that enables plants to endure recurrent harvesting by effectively restoring their photosynthetic capacity and sustaining the integrity of the basal region. A higher number of tillers reduces apical dominance, which in turn encourages the growth of shoots from the plant's base (Ernawati et al., 2023). Therefore, the observed increase in tiller numbers during the second and third cutting cycles, especially in *U. ruziziensis,* can be attributed to

Table 5
The chemical compositions of U. ruziziensis, Mulato II, U. decumbens and U. brizantha before and after fermentation

Treatment	DM (%)	CP (%)	Ash (%)	EE (%)	NDF (%)	ADF (%)	ADL (%)	IVTDMD (%)	NDFD (% NDF)	pH	NH3-N (%)
Before fermentation											
U. ruziziensis	24.06$^{b1/}$	9.56	10.53	1.60^{ab}	67.00^{ab}	31.73^{c}	4.03^{ab}	70.97^{a}	54.97^{a}	5.56^{b}	0.006^{a}
Mulato II	24.00^{b}	6.93	12.17	1.63^{a}	64.30^{b}	32.03^{bc}	3.90^{b}	71.90^{a}	61.40^{a}	5.76^{ab}	0.006^{a}
U. decumbens	29.53^{a}	7.60	10.80	1.43^{b}	68.40^{ab}	34.07^{ab}	4.20^{a}	60.23^{b}	45.57^{b}	5.81^{ab}	0.006^{a}
U. brizantha	27.67^{a}	7.16	10.36	1.50^{ab}	69.53^{a}	34.96^{a}	4.13^{a}	64.57^{b}	52.87^{ab}	5.90^{a}	0.004^{b}
F-test	**	ns	ns	*	**	*	**	**	**	**	*
C.V. (%)	4.04	15.56	11.65	6.02	2.14	3.38	1.78	2.98	5.45	0.84	10.18
After fermentation											
U. ruziziensis	18.47^{b}	10.00	14.43^{b}	2.90^{ab}	60.87^{bc}	36.47^{b}	5.03^{b}	71.09	38.41	4.56^{ab}	0.13^{ab}
Mulato II	18.67^{b}	9.77	20.17^{a}	3.23^{a}	57.10^{c}	34.73^{b}	4.47^{b}	71.75	38.64	4.57^{ab}	0.16^{ab}
U. decumbens	23.07^{a}	8.33	15.33^{b}	2.17^{c}	67.00^{a}	42.73^{a}	6.33^{a}	70.03	37.79	4.67^{a}	0.17^{a}
U. brizantha	22.23^{a}	7.80	14.16^{b}	2.30^{bc}	64.00^{ab}	38.97^{ab}	5.07^{b}	69.18	35.72	4.46^{b}	0.15^{b}
F-test	**	ns	*	*	*	**	*	ns	ns	**	*
C.V. (%)	4.49	14.47	7.93	12.11	3.05	4.62	10.51	2.96	12.60	1.06	11.86

Note. ns = non-significant, * = significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$)
[1/]Means in a column with the same letter are not significantly different based on least significant difference (LSD)

an adaptive response aimed at enhancing resilience to recurrent harvesting. *U. brizantha* and Mulato II exhibited a reduction in tiller numbers during the third cutting cycle, potentially because of harvesting-induced damage sustained in the preceding cycle.

The variation in leaf greenness via SPAD values further suggests the importance of nutrient management strategies to optimise photosynthetic efficiency within *Urochloa* species (Pinho Costa et al., 2014). Silva et al. (2018) reported that chlorophyll content declines with increasing plant age, in contrast to other agronomic traits that generally exhibit a positive correlation with plant maturity. This observation is consistent with the findings of the present study, which demonstrated a decline in leaf greenness during the third cutting cycle relative to the first, suggesting a reduction in chlorophyll content with plant maturation. However, *U. brizantha* and Mulato II maintained significantly higher levels of leaf greenness, suggesting a greater capacity for chlorophyll retention within the leaf tissues. *U. decumbens* displayed intermediate agronomic traits, underperforming *U. brizantha* and Mulato II in most parameters while exhibiting slightly superior traits to *U. ruziziensis*. These findings indicated the potential of *U. brizantha* as a high-yield, resilient forage crop for Thailand, while Mulato II is identified as a viable alternative under specific management practices to mitigate regrowth limitations (Hare et al., 2005; Hare et al, 2015). However, in terms of consistency from the first to third cutting, *U. brizantha* exhibited higher agronomic traits and some traits and significant differences compared to Mulato II. Agronomic performance showed the potential of *U. brizantha* utilisation in Thailand conditions.

These growth patterns confirm the strong potential of *U. brizantha* for cultivation in Thailand. These results suggest that mechanical cutting drops apical auxin levels, triggering basal bud growth to rebuild the canopy. The varieties handle this stress differently through distinct resource allocation strategies: *U. brizantha* prioritises rapid vertical growth and upper-leaf chlorophyll retention to maximise light capture, whereas *U. ruziziensis* invests its carbohydrate reserves into horizontal tiller expansion rather than height. This may be explained by the fact that these diverse structural pathways illustrate how internal resource routing dictates the regeneration speed of tropical perennial grasses. (Ernawati et al., 2023; Perini et al., 2023).

The fresh and dry weight yields of *Urochloa* grasses varied significantly in three cutting cycles. In the first cutting, *U. brizantha* showed the highest fresh and dry weight yields (4,212.50 and 945.80 kg ha^{-1}, respectively); there were no significant differences compared to Mulato II and *U. decumbens* (Table 2). This suggests that these varieties exhibit comparable early growth potential. The dry matter (DM) did not show significant differences among the varieties in the first cutting, indicating similar forage quality at this stage. In the second cutting, fresh weight and dry weight differed significantly among the varieties. Mulato II exhibited the highest fresh weight (7,560.89 kg ha^{-1}), with no

significant difference compared to *U. decumbens* (6,781.94 kg ha^{-1}) and *U. brizantha* (6,359.65 kg ha^{-1}). For dry weight, *U. decumbens* (1,864.09 kg ha^{-1}) and Mulato II (1,794.21 kg ha^{-1}) give the highest dry weight, while *U. decumbens* exhibited the highest DM content (Table 2). This highlights *U. decumbens* as an excellent candidate for forage conservation methods like silage or hay production. (Singh & Singh, 2017).

By the third cutting, significant differences were observed in both fresh and dry weights among the varieties. *U. brizantha* exhibited the highest fresh weight (16,670.83 kg ha^{-1}) and dry weight (4,520.83 kg ha^{-1}) (Table 2), demonstrating superior regrowth potential and biomass accumulation. However, no significant differences in DM were observed, suggesting that all varieties maintained relatively stable dry matter proportions, which is a crucial aspect of forage nutritional quality (Jank et al., 2014). Crop growth rate is an important factor influencing overall forage yield. The results indicated no significant difference in CGR during the first phase (CGR1), suggesting that the initial growth rates of the varieties were comparable. However, in the second phase (CGR2), significant differences emerged, with *U. brizantha* exhibiting the highest CGR (10.35 kg m^{-2}day^{-1}), followed by *U. ruziziensis*, *U. decumbens*, and Mulato II (Table 3). This suggests that *U. brizantha* has a higher biomass accumulation rate under sustained cutting, making it a suitable option for intensive forage production systems (Thaiana Rueda da Silva et al., 2020).

The rapid recovery and superior daily growth rates of *U. brizantha* under continuous harvesting stem from its deep root system and robust non-structural carbohydrate reserves. After defoliation, this bunchgrass relies on energy stored in its stem base to drive new vegetative shoots. Its extensive root framework extracts moisture and minerals from deeper sandy soil layers even during surface dry spells, maintaining the cellular turgor necessary for continuous cell division. This sustained metabolic activity prevents post-harvest stagnation, matching recent field trials where deep root depth and quick energy mobilisation directly sustained tropical pasture regrowth speeds (Dereje et al., 2024; Thaiana Rueda da Silva et al., 2020).

The nutritional analysis of the four *Urochloa* grass varieties within three cutting cycles provides implications for livestock feed management. In the first cutting, crude protein was consistent among the four varieties, ranging from 10.36% to 11.36% (Table 4) and decreased in the second and third cutting, which corresponds to the study conducted by Rodríguez et al. (2024), which reported a decline in crude protein content with advancing plant maturity across six *Urochloa* hybrids. Moreover, the reduction in crude protein content may be associated with the decline in leaf greenness, as the SPAD index has been shown to serve as a reliable indicator of protein levels in plants (Silva et al., 2018). This study revealed that the concentrations of NDF and ADF increased with plant maturity due to the progressive development and thickening of cell wall structures (Jung & Allen, 1995).

However, *U. decumbens* exhibited the highest levels of NDF, ADF, and lignin, indicating that it is more fibrous and potentially less digestible than other varieties (Vinhas Itavo et al., 2022). On the other hand, *U. brizantha* provided the highest EE levels, suggesting it may offer higher fat content, which can contribute to energy intake in livestock diets (Ponnampalam et al., 2024). Ash levels were similar among *U. ruziziensis*, Mulato II, and *U. decumbens*, indicating a comparable mineral composition in these varieties.

During the second cutting, CP levels declined to a range of 7.4% to 8.4% (Table 4) with no significant differences among varieties. This reduction in protein content is associated with the expected decline in nutrient concentration as grasses mature (Bantihun et al., 2022). Similarly, ash content increased to a range of 11.00% to 14.26% (Table 4) without significant differences among varieties, suggesting consistent mineral content despite the decline in protein levels. The most notable differences during this cycle were observed in the fibre components. *U. ruziziensis* and Mulato II had significantly lower NDF levels than other varieties, indicating higher digestibility (Dereje et al., 2024). In contrast, *U. decumbens* maintained the highest levels of ADF and ADL, reinforcing its more fibrous nature. Furthermore, *U. ruziziensis* and Mulato II exhibited higher EE levels, suggesting a potential advantage in energy content during this growth phase. In the third cutting, CP, ADF, and ADL levels remained statistically similar among varieties, indicating that nutrient composition may stabilise as the grass matures. Nevertheless, *U. ruziziensis* demonstrated the lowest NDF levels, suggesting its potential for higher digestibility in later stages (Dereje et al., 2024). Ash content was significantly higher in *U. ruziziensis* and Mulato II, suggesting greater mineral availability in these varieties (Bantihun et al., 2022). Notably, *U. decumbens* gave the highest EE levels, indicating a relatively high fat content that may enhance its energy contribution to livestock feed (Ponnampalam et al., 2024).

This drop in crude protein combined with rising NDF and ADF values stems from plant maturation and a shifting leaf-to-stem ratio. As the grass ages, it redirects resources from producing soluble cell contents, like metabolic proteins and enzymes, toward structural stem growth. To support this vertical weight, the plant builds thick layers of cellulose, hemicellulose, and complex lignin within its cell walls. *U. decumbens*, for example, exhibits rapid stem lignification, accumulating structural carbohydrates into vascular bundles at the expense of nitrogenous leaf proteins. This trend mirrors classic forage studies where advancing maturity accelerates cell wall development while diluting overall crude protein content. (Jung & Allen, 1995; Rodríguez et al., 2024).

U. decumbens and *U. brizantha* demonstrated significantly higher DM content relative to the other grass varieties, both before and after ensiling. The sustained DM content in these grasses indicates a superior capacity to retain dry matter throughout the fermentation process, a critical factor in preserving nutrient integrity in silage. However, CP levels showed no significant variation among all varieties, suggesting that the ensiling process had

a minimal effect on protein retention. This observation aligns with prior research indicating negligible CP alterations post-ensiling in tropical grasses (Sath et al., 2013). Interestingly, the ash content of Mulato II significantly increased after fermentation, indicating a potential accumulation of mineral components during the fermentation process. These results may be attributed to the mineral composition levels of the substrates and indicate the effectiveness of the microbial consortium in improving the mineral composition of the substrates (Ntuli et al., 2013). Such a finding is consistent with reports on tropical forages, where fermentation processes can influence ash concentrations (Ogodo et al., 2017).

The pH and NH_3-N concentrations exhibited significant variations among the grass varieties, suggesting that the impact of grass species on fermentation quality. *U. brizantha* demonstrated the highest pre-fermentation pH, suggesting lower fermentable carbohydrate availability or a higher buffering capacity before ensiling. On the other hand, *U. decumbens* showed the highest post-fermentation pH, potentially indicating suboptimal fermentation efficiency. The observed NH_3-N concentrations in *U. brizantha* were significantly lower in both pre- and post-fermentation stages, suggesting better protein preservation and reduced proteolytic activity in this grass variety. Assessment of digestibility through IVTDMD and NDFD revealed that *U. ruziziensis* and Mulato II demonstrated superior fibre digestibility before ensiling, consistent with findings reported in other tropical forage studies (Jayasinghe et al., 2022). However, after fermentation, analysis indicated no significant differences in digestibility among the grass varieties, suggesting that the fermentation process may attenuate initial variations in fibre composition and digestibility (Zhang et al., 2017). The lack of significant NDFD differences after ensiling is particularly significant, as it emphasises the potential of the fermentation process to homogenise digestibility traits throughout ensiled forages, effectively minimising variability in fibre degradability. Previous studies indicate that ensiling can mitigate initial differences in fibre composition through microbial degradation and structural breakdown (Oba & Allen, 2000).

The excellent protein protection in *U. brizantha* results from a fast drop in pH caused by early organic acid accumulation under anaerobic conditions. This rapid acidification blocks plant proteases, stopping the breakdown of true proteins into ammonia and volatile nitrogen. Concurrently, during the 21-day storage period, lactic acid bacteria carry out natural microbial hydrolysis to partially break down structural hemicellulose bonds across all varieties. This cellular breakdown erases the natural differences between the species to create a uniform fibre product. This aligns with findings that effective fermentation standardises dry matter digestibility in tropical silage. (Alves et al., 2025; Ponnampalam et al., 2024).

In conclusion, the four *Urochloa* species exhibited distinct agronomic and nutritional characteristics under Thailand's tropical conditions. Among the evaluated species, *U. brizantha* showed the greatest potential for intensive forage production and repeated

cutting systems due to its superior biomass yield and growth performance. *U. ruziziensis* demonstrated favourable digestibility characteristics, indicating its suitability for silage production, while *U. decumbens* exhibited relatively high dry matter content and stable performance, suggesting potential for forage production under drought-prone conditions. These findings indicated the importance of selecting forage species according to specific production objectives and provide practical guidance for forage management, year-round feed production, and silage utilisation in Thai livestock production systems. Future research should further investigate the effects of seasonal variability and ensiling practices on forage quality and fermentation characteristics to optimise sustainable livestock feeding strategies in tropical environments. The study indicated the significance of selecting grass varieties with superior agronomic performance, high yield and digestibility potential to enhance the nutritional quality of silage. Future research should explore the interaction between ensiling conditions and grass variety, particularly in tropical regions where climatic variability can significantly affect fermentation dynamics and nutrient preservation. This approach will provide important information for optimising forage management strategies and promoting sustainable livestock feeding systems.

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Effect of Shading and Water Stress on the Vegetative and Generative Growth Patterns of Arrowroot (*Maranta arundinacea* L.)

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ABSTRACT

Arrowroot (*Maranta arundinacea* L.) plants have distinct vegetative and generative growth patterns under shading and water stress. This current study aimed to evaluate the growth patterns of arrowroot plants under various shading and water availability conditions. Two factors, shading levels and water availability, were set as treatments. Four shading levels were 0%, 25%, 50%, and 75%. Water availability treatments were inundation for 5, 10, and 15 days, and watering intervals of 5, 10, 15, and 20 days. A nested design with the watering factor nested in the shading factor was used to arrange the treatments. All treatments were repeated three times. The findings revealed that maximum vegetative growth occurred eight months after planting. While the growth of the generative phase increased in older plants up to ten months following planting. Plant vegetative growth was significantly influenced by shading treatment, whereas tuber growth was unaffected. The inundation treatments enhanced root number and plant biomass, while drought stress inhibited vegetative and generative growth. The 15-day inundation treatment did not significantly affect plant growth; however, growth was enhanced following the stress period. In contrast, drought stress over 20 days caused considerable plant growth retardation. The arrowroot plants can be grown under 50-70% shading and water stress for 10 days.

Keywords: Drought, growth, inundation, light, *Maranta*

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INTRODUCTION

Arrowroot is one of the underutilised tuber crops which is cultivated beneath forest canopy or in residential gardens. The tubers

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are commonly used in food and traditional medicine because of their starch content (Aprianita et al., 2014; Deswina & Priadi, 2020). Arrowroot tubers are used as raw materials for meals (Malki et al., 2023) and health products in industrial sectors (Priatama et al., 2019). Maturity of plants and environmental factors during planting influence production and quality of arrowroot. According to Enesi et al. (2022), favourable environmental conditions promote tuber development and productivity, whereas suboptimal conditions may reduce yield and quality of arrowroot. Understanding the optimal growth of arrowroot is critical for obtaining the optimal growth of the crops.

Studies on the growth pattern of arrowroot crops can indicate the ideal period for collecting the tubers. Vegetative and generative growth are two main growth phases of arrowroot plants. Vegetative growth focuses on the development of roots, stems, and leaves, whereas generative growth includes initiation and maturation of tubers (Brito et al., 2019; Yursak et al., 2021). Understanding the growth pattern of arrowroot is important because the allocation of assimilates between vegetative and storage organs changes throughout plant development. Information on growth dynamics can therefore improve our understanding of how environmental factors, such as shading and water availability, influence crop growth and tuber production (Rusmin et al., 2018).

Light is one of the main environmental factors influencing plant growth. Light is essential for photosynthesis. Arrowroot plants produce optimal yield under around 27% shading, while growth and tuber yield reduce under shading of 51% to 72% (Oktafani et al., 2018). According to Supriyono et al. (2017), arrowroot plants produce optimal yield under 51% shading. These results clearly show the optimal light intensity variability for arrowroot growth.

Another environmental factor which influences arrowroot growth is water. The photosynthetic process, metabolism, nutrient transport, and plant growth are supported by water availability. Optimal water availability promotes optimal plant growth and tuber formation. On the other hand, water deficit or flooding decreases growth and tuber formation. According to Arduini et al. (2019) and Han et al. (2021), inundation and drought stresses reduced plant growth and yields by reducing photosynthesis, transpiration, stomatal conductance, respiration, and nutrient uptake. Flooding limits oxygen availability, and water stress promotes water deficit in plants (Akhtar & Nazir, 2013). In response to water excess, plants generate adventitious roots that enhance oxygen flow to the roots (Yamauchi et al., 2019). Previous studies showed that limited water availability reduced crop growth patterns, for example in rice and wheat (Seleiman et al., 2021; Zhang et al., 2018).

Inundation tolerance in crops can be triggered by flooding for a certain duration during specific growth stages, while drought stress can be induced by altering irrigation intervals. Lin et al. (2006) demonstrated the efficiency of a seven-day flooding interval as a selection tool for sugar beet tolerance. Inundation for 12 days at 105 and 165 days after planting was

an effective screening tool for cassava plants (Kerddee et al., 2021). Irrigation intervals of once every seven days reduce the growth and tubers of taro and konjac (Santosa et al., 2004; Hidayatullah et al., 2020a). Each crop exhibits a different response to drought and inundation stresses. Despite extensive studies on various commercial crops, research on the effects of shading, inundation, and drought stresses on the growth patterns of arrowroot plants is still limited (Zhao et al., 2022). Our previous study solely focused on the arrowroot plant growth tolerance to shading and watering in a short period after one month of the treatments; however, it had not yet explored the growth pattern (Sutrisno et al., 2023). Therefore, the objective of this study was to examine the long-term effect (6-10 months after planting (MAP)) of shading, drought, and inundation on the growth patterns and yield of arrowroot plants.

MATERIALS AND METHODS

Experimental Site and Treatments

The research was conducted at Jambegede Experimental Station, Kemiri Village, Kepanjen District, Malang, East Java, Indonesia (8°10′31″ S, 112°33′36″ E, 330 m). Briefly, the experiment consisted of two factors, that shading level as the first factor and watering level as the second factor. The shading levels were (A1) 0%, (A2) 25%, (A3) 50%, and (A4) 75%, while the watering levels were inundation for (B1) 15 days, (B2) 10 days, and (B3) 5 days, watering intervals of (B4) 5 days as a control, (B5) 10 days, (B6) 15 days, and (B7) 20 days. The treatments were arranged in a nested design with the watering factor nested in the shading factor, with three blocks, resulting in 84 experimental units. The planting distance was set at 40 cm × 40 cm (Anggun et al., 2017). Each experimental unit consisted of 15 polybags with drainage holes at the bottom to facilitate drainage and allow water movement during the inundation treatments.

Propagation Material and Planting Media Preparation

The planting media was prepared for the nursery (7 cm × 12 cm polybag) and treatment media (40 cm × 40 cm polybag). Uniform sizes of arrowroot cuttings were grown in nursery media for six weeks to ensure proper cutting development before moving on to treatment media. Arrowroot plants grown on the treatment media were maintained until harvest. Soil, rice husks, and manure (5:2:1 v/v/v) were used as nursery and treatment media, with one plant per polybag (Sutrisno et al., 2023).

Application of Shading and Waterlogging Stress

Environmental modification was achieved using four separate plastic houses. The control (0% shading) was maintained in a plastic house without additional shading nets. The other

three plastic houses were covered with commercial high-density polyethene plastic nets, providing shading levels of 75%, 50%, and 25%. The plastic nets were installed below the plastic house at 2 meters above the ground level. Furthermore, plastic nets were installed around the plastic house to permit light to enter the plastic house during the morning, afternoon, and evening hours, according to the shading levels (Sutrisno et al., 2023).

Watering treatment was conducted on arrowroot plants placed in a pond constructed using plastic-layered wooden planks. The watering was performed according to the specified treatments, which were started two weeks after transferring the plants to the plastic house (Sutrisno et al., 2023).

Growth Observations and Final Harvest Procedures

Throughout the time of observation of 6 to 10 MAP, growth metrics were measured, including stem diameter, stem weight, plant height, number of tillers, number of leaves, leaf area and weight, as well as number, length, weight, and volume of roots. Length, diameter, and weight of tubers were measured at harvesting.

The combined weight of the primary stem and its descendants per plant was calculated as total stem weight. A freely available ImageJ software was used to calculate total leaf area per polybag. The volume of the roots was calculated using a volumetric flask in which, after immersing the roots in water, elevated water volume in the container after the root immersion was the root volume (Sutrisno et al., 2023). The number of saplings grown around the main stem was summed as the number of tillers.

Methods of Data Analysis and Interpretation

The mean value of each parameter at each observation time was plotted to figure the growth pattern from 6 to 10 MAP. The peak growth point represented the maximum growth of each character, which could then be used as a reference point for determining the optimal harvesting time for arrowroot plants grown under different shading and inundation conditions. Graph data were presented as means ± the standard error based on three replications. Statistical analyses were performed using R 4.5.1 (R Core Team, 2025). Analysis of variance was conducted separately for each observation period to evaluate the effect of shading, watering treatments, and their interaction on the observed parameters. Differences among treatments were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Assessment of Vegetative Parameters

The shading intensity significantly affected plant height throughout the observation period ($P < 0.001$), while watering treatments significantly affected plant height at all observation

times. No significant interaction between shading and watering treatments was observed. From 6 to 9 MAP, the plant height increased by 18%. The shading intensity enhanced plant height. The lowest plant height was achieved from arrowroot plants cultivated under 0% shading, whereas the highest was observed from plants grown under 50% shading, except for 9 MAP, where the height was slightly lower than plant height under 75% shading. Similar plant height was achieved under 50% and 75% shading levels at 9 and 10 MAP. Inundation increased plant height; in contrast, drought stress inhibited this parameter. The inundation treatments between 5- and 15-days increased plant height by 2%, whereas the drought decreased arrowroot plant height by 8% compared to the control (Figure 1).

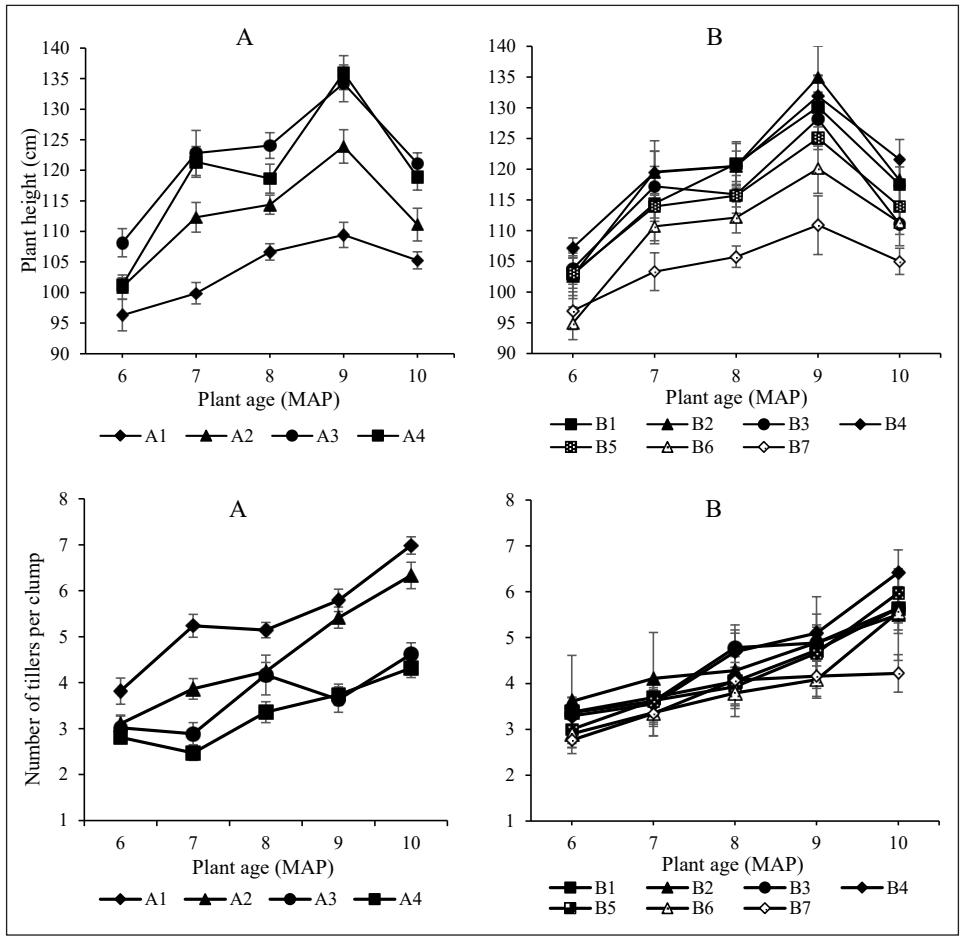


Figure 1. Arrowroot plant height and number of tillers at different plant ages under four shading levels (A) and seven watering levels (B)

Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting. Bars represent the standard error

The maximum increase in plant height up to 9 MAP and subsequent reduction at 10 MAP suggested that the vegetative growth of arrowroot reached a maximum at 9 MAP and declined thereafter. The plants then enhanced the generative phase to optimise tuber enlargement. Prioritising growth from the vegetative to the generative phases has also been reported in cassava (Phoncharoen et al., 2019). The increased growth under shading conditions was due to the reduced light intensity, prompting plants to accelerate height growth to maximise light energy acquisition (Kalaitzoglou et al., 2019). The higher plant growth under inundation than drought stress was because the drought-stressed plants received limited water, thus unable to maximise plant height (Zhen et al., 2019).

The number of tillers per clump increased from 6 to 10 MAP. Analysis of variance showed that shading significantly affected the number of tillers throughout the observation period, whereas watering treatments significantly affected this parameter only at 10 MAP. At 6 MAP, the number of tillers ranged from 2 to 4 tillers per clump, while at 10 MAP, the number of tillers ranged from 4 to 7 tillers per clump, with an increase of one tiller per clump per month. The shading intensity decreased the number of tillers per clump. The highest number of tillers per clump was produced by arrowroot plants under 0% shading, while the lowest was under 75% shading. The effect of watering intervals on the number of tillers varied. The same number of tillers was produced by plants treated with 15 days of inundation and 15 days of drought. However, treatment of 20 days of drought stress produced the lowest number of tillers. The number of tillers under the 20-day watering interval treatment did not increase (4 tillers) at 8 to 10 MAP, while in the other treatments, the number increased by 5-6 tillers (Figure 1). This finding suggested that arrowroot continued to experience vegetative growth alongside the process of tuber formation and enlargement. Some plant species that propagate through tillers produce numerous tillers when actively forming tubers (Hamaoka et al., 2022). The higher number of tillers under shading suggested that plants were more adaptable to shading conditions than open areas (Sudrajat et al., 2023). The increase in the number of tillers was greater under inundation than under drought stress, as water deficiency inhibited the growth of new tillers in plants (Hidayatullah et al., 2020b).

Statistical analysis indicated that shading significantly affected leaf number only at 10 MAP, whereas watering treatments significantly affected leaf number at 9 and 10 MAP. A significant interaction between shading and watering treatments was observed at 8 and 9 MAP. The highest number of leaves was produced at 8 MAP, with an increase of 36% compared to the number of leaves produced at 6 MAP. However, the number of leaves then decreased by 14.6% at 9 and 10 MAP. Shading intensity suppressed the number of leaves. At 8-10 MAP, 0% shading yielded the highest number of leaves, while 50% and 75% shading levels produced the fewest. Watering treatments also affected leaf number, with drought stress causing a greater reduction than inundation. At 8-10 MAP, inundation for 15 and 10 days reduced the number of leaves by 13-23%, while drought for 15-20 days

suppressed the leaf number by 24-50% in comparison to the 5-day irrigation interval or the control (Figure 2).

The maximum increase in the number of leaves at 8 MAP and the subsequent decrease at 9-10 MAP suggested that arrowroot plants maximised the number of leaves until 8 MAP and then started to reduce leaf number at 9-10 MAP to enhance their generative growth (Khalid et al., 2019). The reduction of leaf number due to shading was a consequence of plant adaptation to the limited light conditions by reducing leaf number while attempting to maximise their generative growth with the available light (Fan et al., 2018). Inundation reduced leaf number because plants were stressed by oxygen shortage, which inhibited plant metabolism and the formation of new leaves (Huang et al., 2022). Meanwhile, drought stress reduced the number of leaves due to water deficiency to support photosynthesis and plant growth (Mathobo et al., 2017).

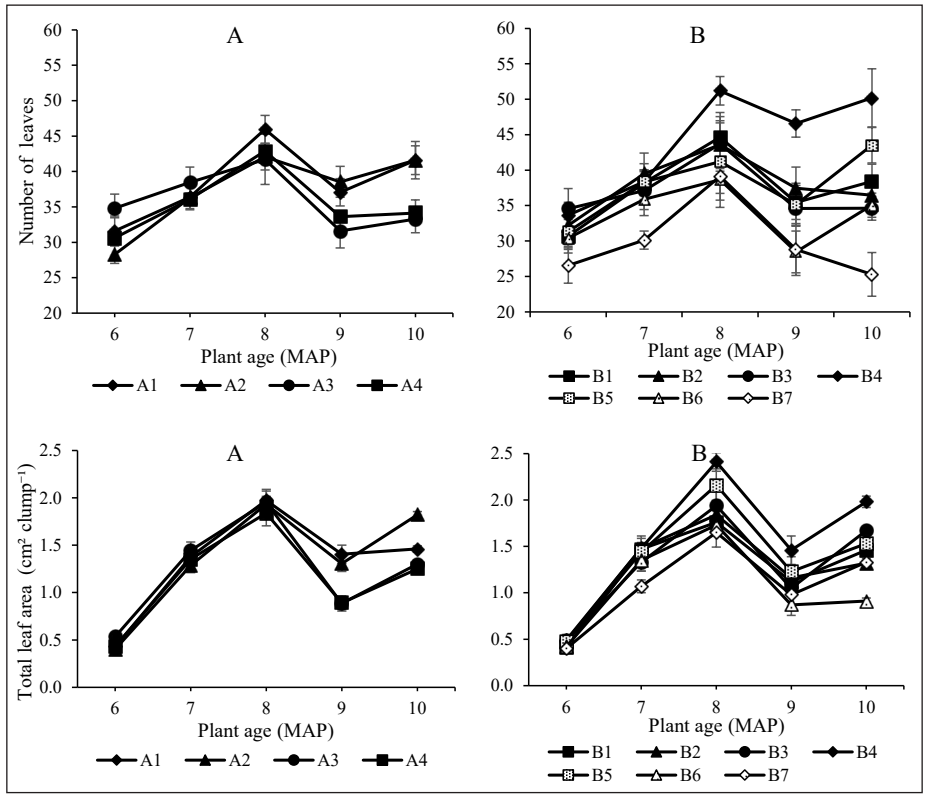


Figure 2. Number of leaves and total leaf area per clump at 6-10 MAP under four shading levels (A) and seven watering levels (B)

Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting. Bars represent the standard error

Shading significantly affected total leaf area at 6, 9, and 10 MAP, whereas watering treatments significantly affected total leaf area from 8 to 10 MAP based on the analysis of variance. A significant interaction between shading and watering treatments was observed at 8 and 9 MAP. The increase in total leaf area per clump by approximately 400 % occurred from 6 to 8 MAP, and decreased by 40 % at 9-10 MAP. Treatments of 0% and 25% shading produced larger total leaf area than 50% and 75% shading. At 10 MAP, the treatment of 0% shading showed the highest total leaf area, while 75% shading produced the lowest total leaf area. Watering stress suppressed total leaf area. A 15-day period of inundation at 8-10 MAP resulted in 22-28% total leaf area reduction, while a 15-day drought stress reduced 31-54% total leaf area compared to the control (Figure 2).

The maximum increase in total leaf area at 8 MAP was related to the increase in the number of leaves during this period. The number of leaves correlated to the size of the total leaf surface area. The lower shading levels, particularly 0% and 25%, produced a larger total leaf area because the plants had more leaves than 50% and 75% shading levels. Inundation treatments resulted in a larger leaf area than drought stress because inundation produced more leaves than drought stress. The increase in total leaf area due to the number of leaves was also reported by Supriyono et al. (2017).

Shading significantly affected stem fresh weight at most observation periods, whereas watering treatment significantly influenced this parameter except at 8 MAP. Stem fresh weight reached the maximum at 8 MAP and declined at 9 to 10 MAP. Between 6 and 8 MAP, the fresh weight was increased by 36%, while between 8 and 10 MAP, the weight decreased by 33%. Shading intensity suppressed stem fresh weight. From 7 to 10 MAP, the stem fresh weight under 50% and 75% shading levels decreased by 17-35% compared to 0% and 25% shading levels. Meanwhile, water stress suppressed the stem fresh weight. At 6 MAP, 15 days of inundation suppressed the stem fresh weight by 8%, while drought stress for 15 and 20 days suppressed the fresh weight by 29-43%. At 10 MAP, the inundation suppressed the stem fresh weight by 15-25%, while drought stress for 15 and 20 days also suppressed this parameter by 17% and 35% (Figure 3).

The increase in the stem fresh weight at 6-8 MAP was because the plants were actively engaged in vegetative growth. At 9-10 MAP, the plants diverted to the generative phase, resulting in the reduction of stem fresh weight. The plants turned senescent, as shown by wilting and weakening stems. The decreased stem weight of the plants under shading because light limitation inhibiting vegetative growth (Liu et al. 2018). The significant stem weight decreases under drought conditions compared to inundation could be due to plant recovery following a period of flooding. In contrast, drought stress causes limited water supply, therefore reducing stem growth (Creek et al., 2018).

Leaf fresh weight increased from 6 to 8 MAP and reached the maximum value at 8 MAP before declining. Between 6 and 8 MAP, the leaf fresh weight increased by 26%

and decreased by 36% at 10 MAP. Shading intensity suppressed the leaf fresh weight as observed from 8 MAP onwards, whereas watering treatments significantly affected this parameter at 8-10 MAP. The decrease in leaf fresh weight under shading levels between 0% and 75% reached 26%. Water stress suppressed the fresh leaf weight from 6 to 10 MAP. At 6, 8, and 10 MAP, inundation reduced leaf weight by 15%, 22%, and 35%, respectively, while drought stress reduced the leaf weight by 29%, 25%, and 49%, respectively. This finding confirmed that drought stress caused a greater reduction in leaf fresh weight than inundation (Figure 3).

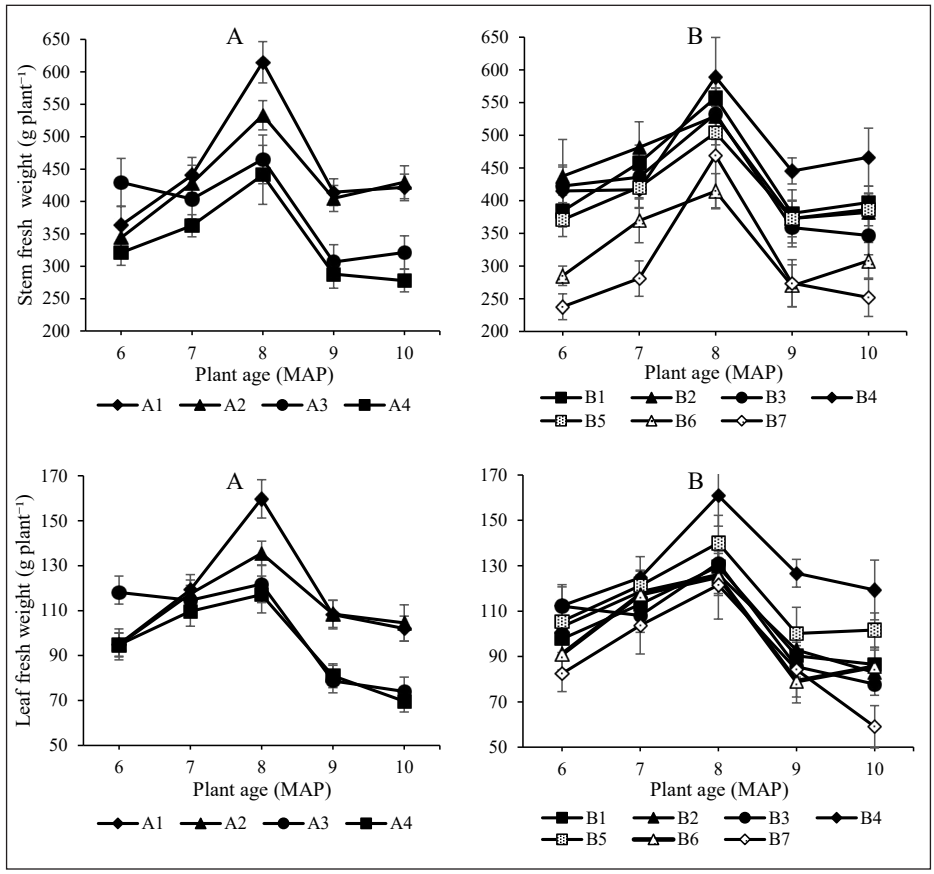


Figure 3. Stem and leaf fresh weight at 6-10 MAP under four shading levels (A) and seven watering levels (B)
Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting

The gradual increase in leaf fresh weight at 8 MAP and the subsequent decrease at 10 MAP could mean the plants were actively maximising vegetative growth at 8 MAP, while at 10 MAP, the plants shifted to generative growth. The greater decrease in leaf

fresh weight under higher shading intensities because the plants reduced vegetative growth under limited light conditions. The greater decrease in drought compared to inundation was because the plants showed recovery under the inundation treatments; on the other hand, the growth pressure continued under drought stress (Creek *et al.,* 2018).

The number of roots increased by approximately 48% in the older plants between 6 and 10 MAP. Both shading and watering treatments affected root number significantly, whereas the interaction between the two factors was significant at 9 MAP. Shading intensity suppressed the number of roots. At 6 MAP, the difference in root number under shading treatments was relatively small; however, from 7 to 10 MAP, the treatments of 0% shading produced the highest root numbers, while plants under 75% shading produced the fewest. Plants under 25% shading also yielded higher root numbers compared to the 50% and 75% shading intensities. At 7 MAP, 25-75% shading levels reduced the root number by 14%, 34%, and 46%, respectively, while at 10 MAP, the root numbers decreased by 2%, 32%, and 39%, respectively. Inundation treatments increased the root number, while drought stress had the opposite effect of reducing the root number. The average number of roots under inundation increased by 7% at 7 MAP, while drought stress reduced the number by 17%. The average number of roots under inundation and drought increased by 20% and decreased by 26%, respectively, compared to the control at 9 MAP. The highest number of roots was produced by plants under inundation for 10 and 15 days, while the lowest was produced by plants under drought stress for 20 days (Figure 4).

The increase in the number of roots between 6-10 MAP showed that the plant roots continued to grow. This increase was an effort of the plant to enhance water and nutrient uptake to meet the plant metabolic needs. The increase in vegetative growth, particularly the upper parts of the plant, such as the stems and leaves, was in line with the increase in root numbers. This occurred in both 0% and 25% shading intensities as well as under inundation treatments. Furthermore, a positive correlation between the increase in root number and stem and leaf growth has been reported in maize crops (Guo et al., 2021).

The fresh weight of roots increased with the older arrowroot plants. Shading influenced root fresh weight at all observation periods, whereas watering treatments affected it from 8 to 10 MAP. At 6 MAP, the root fresh weight ranged from 28 to 92 grams, while at 10 MAP, the weight ranged from 40 to 220 grams, representing an increase from 40 to 139%. Shading levels suppressed the root fresh weight. At 6 MAP, the root fresh weight under a 75% shading level decreased by 30%, while at 10 MAP it experienced a significant decrease by 81% compared to the 0% shading level. Inundation treatments increased the root fresh weight; on the other hand, drought stress decreased this parameter. At 6 MAP, the root fresh weight under inundation was similar to the control, while the root weight under drought stress was slightly lower than the control. At 10 MAP, inundation increased the root fresh weight by 55%; in contrast, this parameter under drought stress decreased by 67% (Figure 4).

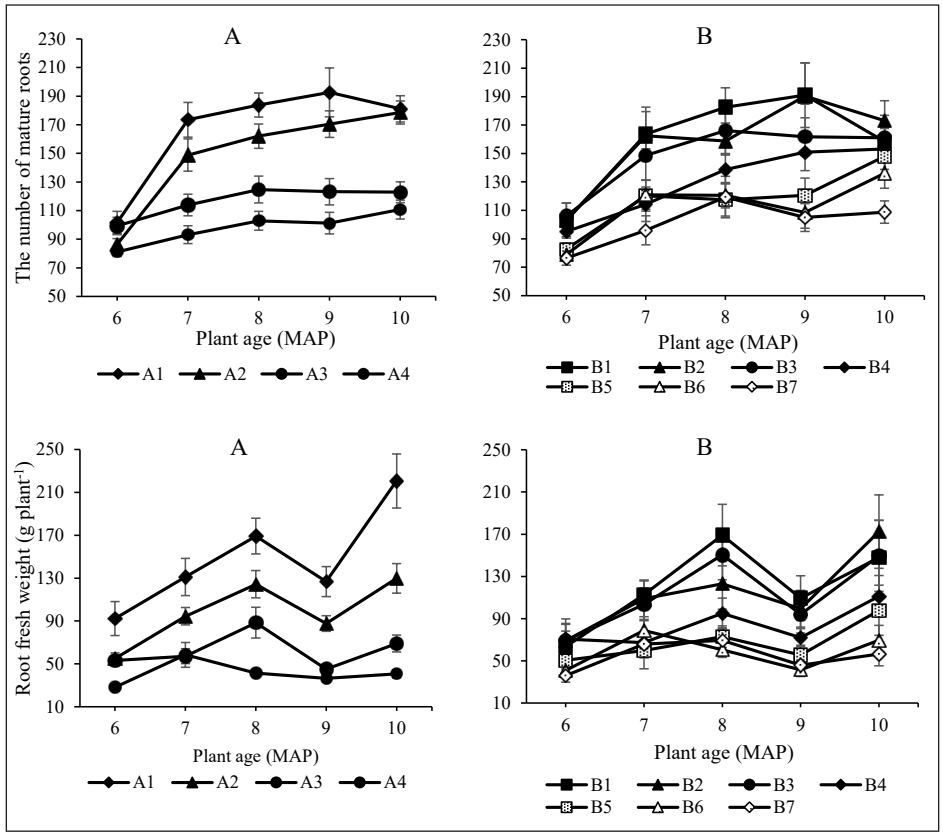


Figure 4. The number of mature roots and fresh weight of roots at 6-10 MAP under four shading levels (A) and seven watering levels (B)
Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting. Bars represent the standard error

The increase in the root fresh weight was due to the plant's requirement for vegetative growth of the leaves and stems. To support leaf and stem growth, the plant developed root growth to supply water and nutrients to the upper parts of the plant and to support the growth of tubers. The decrease in root weight under shading was because of the plant's adaptation to the growth of the upper parts. The shading resulted in slow vegetative growth of the upper part of the plant, thus leading to slow root growth. The same conditions also occurred under inundation and drought treatments (Gaballah et al., 2021).

Assessment of Reproductive Parameters

The older plants showed a significant increase in the number of arrowroot tubers. The number of tubers between 6 and 10 MAP demonstrated a noticeable growth of

approximately 100-120%. Shading levels played a crucial role in regulating the rate of tuber proliferation. From 6 to 10 MAP, the number of tubers under 0% shading was 181% higher than that under 75% shading. Inundation treatments increased tuber quantity. The treatment of inundation for 15 days produced the least tuber quantity, in comparison with 10 and 5 days of inundation at the same time of the observation. The highest number of tubers was observed in the 10-day inundation. It was not surprising that drought stress suppressed the tuber quantity. At 6 MAP, the number of tubers under drought stress was similar to the control; however, from 8 to 10 MAP, the tuber quantity under drought stress was lower. Prolonged drought stress suppressed the tuber quantity, showing the lowest number of tubers under 20-day drought treatment (Figure 5).

The tuber length was not affected by plant age. The length measured was between 16 and 18 cm at 6 to 10 MAP. The shading level had a minor effect on the tuber length.

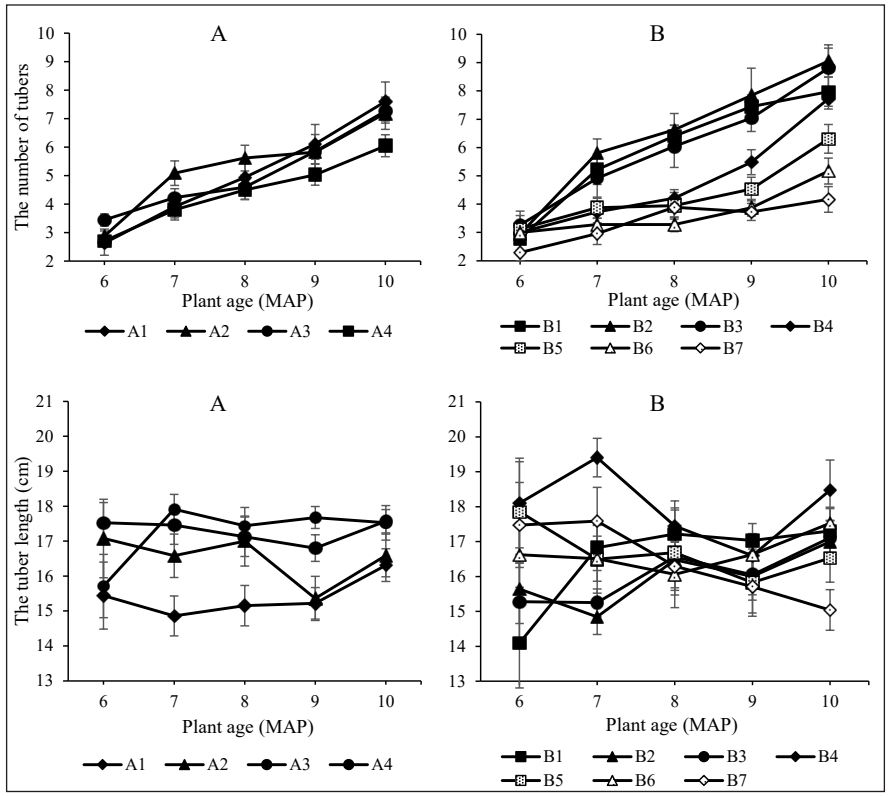


Figure 5. The number and length of tubers at 6-10 MAP under four shading levels (A) and seven watering levels (B)

Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting. Bars represent the standard error

The absence of shading and under 25% shading intensity resulted in shorter tuber length compared to the shading intensities of 50% and 75%, particularly at 7 and 10 MAP. Plants grown without shading had the shortest length, in contrast to the plants grown under 75% shading, which yielded the longest tuber. Furthermore, water stress inhibited tuber length. Drought stress resulted in slightly longer tubers compared to inundation at 6 MAP. However, a comparison at 10 MAP showed that tubers produced under drought stress were shorter than those produced under inundation stress. The 20-day drought stress produced the smallest and the longest tubers. Inundation reduced tuber length by 6%, while drought stress decreased tuber length by 16% (Figure 5).

No significant tuber length variation among treatments from 6 to 10 MAP was observed. This was because the arrowroot plants emphasised tuber length growth, starting from the beginning of tuber development. After 6 MAP, the plants focused on tuber enlargement. The plant's ability to optimise tuber production while minimising vegetative growth may account for the increased tuber size. Conversely, under low shading conditions, there was an increase in plant growth but a decrease in tuber size.

The diameter of arrowroot tubers continually increased until 10 MAP; however, the growth rate was faster at 6 to 8 MAP than from 8 to 10 MAP. The tuber diameters at 6 to 8 MAP increased by 45%, while from 8 to 10 MAP the increase was only 10%. The shading intensity influenced the tuber diameter. From 7 to 10 MAP, the tuber diameter under 0% shading was consistently the lowest compared to the 25-75% shading levels. The tuber diameter under 25-75% shading was similar at all plant ages from 6 to 10 MAP (Figure 6).

The effect of watering stress on the tuber diameter at 6 to 10 MAP was insignificant. However, the effects of inundation and drought treatments differed at various stages of plant development. At 6 to 7 MAP, inundation resulted in the smallest tuber diameter, but this parameter increased from 8 to 10 MAP. Conversely, the drought treatment showed an opposite pattern, with the largest tuber diameter observed in the control (Figure 6).

The elevated increase in tuber diameter at 6 to 8 MAP suggested that the plant was prioritising the expansion of the tuber dimensions. The accelerated growth of the vegetative organs was observed at 6 to 8 MAP and less at 8 to 10 MAP. The decline in vegetative growth and tuber growth rate at 8 to 10 MAP suggested that the tuber dimensions were not the only factor driving plant development at this stage. At 6-7 MAP, the modest tuber diameter increase was most likely due to plant recovery from flooding. After the recovery process, the plants saw accelerated tuber growth at 8-10 MAP. However, water deficit slowed tuber growth at 8-10 MAP, resulting in decreased tuber diameter. This finding was in line with a study conducted by Kengkanna et al. (2019).

The tuber fresh weight increased with the older arrowroot plants. From 6 to 10 months, the tuber fresh weight increased by 380%. Shading affected the tuber fresh weight at all observation times, whereas watering treatments influenced the tuber fresh weight at 6 and 8-10 MAP. The 0% shading level produced the lightest tuber fresh weight compared to the

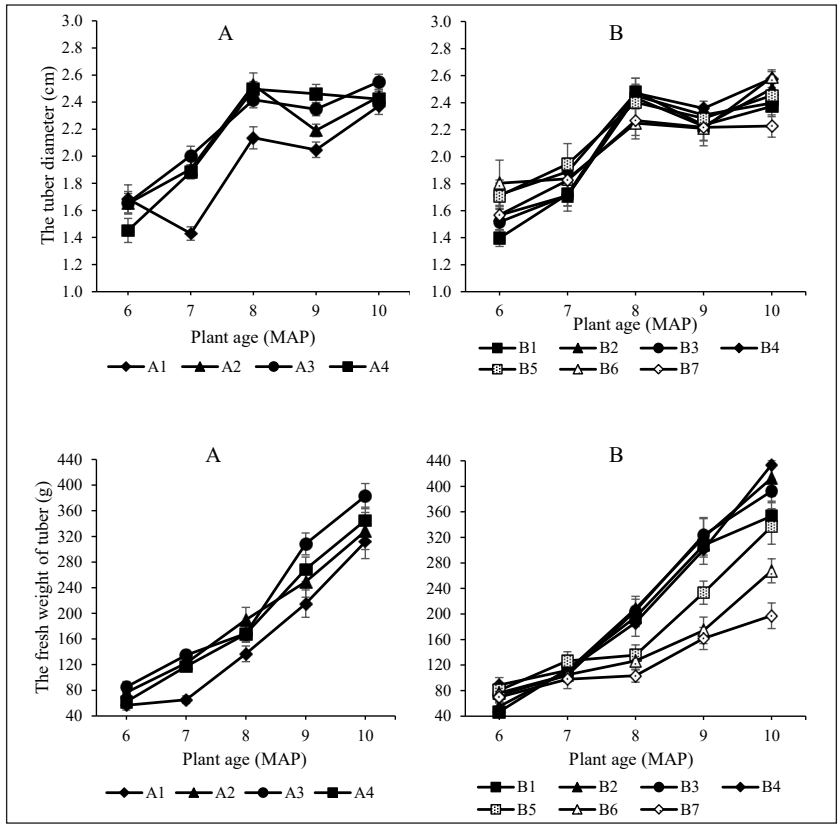


Figure 6. The diameter and fresh weight of tubers at 6-10 months after planting under four shading levels (A) and seven watering levels (B)
Note. (A1) 0% shading, (A2) 25% shading, (A3) 50% shading, (A4) 75% shading, (B1) 15-day inundation, (B2) 10-day inundation, (B3) 5-day inundation, (B4) 5-day irrigation interval, (B5) 10-day irrigation interval, (B6) 15-day irrigation interval, (B7) 20-day irrigation interval, (MAP) months after planting. Bars represent the standard error

25%-75% shading, with the heaviest tuber fresh weight under the 50% shading. Watering treatments affected the increase in tuber fresh weight. The control treatment yielded the heaviest tuber fresh weight, followed by inundation and drought conditions. The tuber fresh weight of the control increased by 360% from 6 to 10 MAP, whereas under inundation and drought conditions, the increases were 300% and 182%, respectively (Figure 6).

The correlation between the heavier tuber fresh weight and the older arrowroot plants (8-10 MAP) suggested that this phase emphasised tuber development. The heavier tuber fresh weight under a higher level of shade suggested that arrowroot plants exhibited higher tolerance to low-light than full light conditions. The increase in tuber fresh weight under inundation showed that the arrowroot plants were more tolerant to waterlogging than drought. Taro was also reported to be tolerant to inundation stress (Hidayatullah et al., 2020b).

The vegetative growth of plant organs, including leaf number, stem weight, leaf weight, and root number, reached the highest at 8 and 9 MAP and started to decline at 9 and 10 MAP. However, the number of stems, tubers, tuber diameter, and tuber weight continued to increase until 10 MAP. Between 9 and 10 MAP, the growth rate of the vegetative organs was slow. However, a notable increase in tuber mass occurred during this period. This finding suggested that the plant shifted its metabolic process from photosynthesis, which was responsible for leaf growth, to tuber growth. Furthermore, the reduced quantity and mass of the leaves suggested that the stored and consumed photosynthetic compounds were reallocated to the tubers (Golovko & Tabalenkova, 2019).

CONCLUSION

The vegetative growth of arrowroot rose until 8 MAP, but tuber development grew during the generative phase until 10 MAP. During the entire observation period, shading influenced the majority of vegetative and generative growth parameters. Although shading lowered several vegetative traits, arrowroot plants could produce substantial tuber formation, demonstrating good adaptation to low-light conditions. Watering treatments had a considerable impact on plant growth and yield components, especially in the late growth phases. Arrowroot plants tolerated inundation for up to 15 days, but prolonged drought stress with a 20-day irrigation interval inhibited considerable growth and tuber production. These findings demonstrate that arrowroot plants are promising underutilised tuber crops for cultivation under shading with fluctuating water availability, where other crops may face significant growth constraints. Further research is required to assess arrowroot response to extended periods of flooding and to determine tolerance mechanisms at various growth stages.

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Endophytic Bacterial Community of Leaf Tissues of *Mangifera indica* L. cv. Harumanis Revealed by 16S rRNA Metagenomics

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ABSTRACT

Numerous studies have demonstrated the importance of plant endophytic bacteria in promoting plant growth and fitness, both of which are crucial to agriculture. They have been shown to enhance nutrient uptake and utilisation, disease resistance, and stress tolerance, resulting in higher yields. However, only a few studies have been carried out on *Mangifera indica* cv. Harumanis endophytes, and those focused on fungi. This study therefore aimed to explore the composition, diversity, and potential functions of the endophytic bacterial community in *M. indica* cv. Harumanis, and this, to the best of our knowledge, is the first report. Fresh and asymptomatic leaves were collected for analysis. The bacterial endophytes were investigated by metagenomic analysis of the V3-V4 region of the 16S rRNA. Results showed unclassified bacteria (51-82%), followed by Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%) as the dominant bacterial phyla in the leaf tissues. Dominant genera revealed in the leaf tissues included *Pseudonocardia, Caulobacter, Nocardioides,* and *Achromobacter,* which are generally considered beneficial in environmental and agricultural contexts. These bacteria possess several plant growth-promoting traits such as nutrient cycling, defence against pathogens

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and stress tolerance, indicating their potential role in sustainable agriculture. The study concludes that the leaves of *M. indica* L. cv. Harumanis have a unique endophytic bacterial community that is crucial for plant growth and health and can be explored for agricultural applications, including biofertilizers and biocontrol agents.

Keywords: Bacterial diversity, folia tissues, fruit crops, plant-microbe interactions, 16S amplicon sequencing

INTRODUCTION

Mangifera indica L., commonly known as mango and belonging to the Anacardiaceae family, is a well-known fruit all over the world. It is economically and culturally valuable, especially in Asia, where it is widely farmed. Mango is one of the most treasured and widely cultivated tropical fruits in the world, with popularity spanning more than 100 nations, particularly in Asia. It was discovered in the Himalayan foothills of Northeastern India, Myanmar, and Bangladesh, and is a popular evergreen fruit tree native to Southeastern Asia (Kumar et al., 2021b). The 'Harumanis' variety, often known as the "King of Mangoes," is mostly grown in Malaysia's northern area, notably in the state of Perlis. It is quite popular in Malaysia with a unique sweetness and fragrant scent (Rathakrishnan et al., 2023). This cultivar is commonly used in Malaysia's commercial mango sector, both for local sale and export. Its distinct flavour and scent distinguish it from other varieties, resulting in considerable demand (Azizan et al., 2019).

Only a few studies have been carried out on *M. indica* cv. Harumanis endophytes, and existing studies are predominantly centred on endophytic fungi, particularly in the context of screening for disease potential and identifying biocontrol agents against various diseases (Ahmad et al., 2023; Bin Rosali et al., 2025; Suhanna et al., 2013; Zainudin et al., 2019). There is limited research conducted on the bacterial endophytes of this plant cultivar. Characterising bacterial endophytes of fruit crops via 16S amplicon sequencing is essential for profiling both culturable and unculturable microbial communities (Alibrandi et al., 2020). This technique helps identify beneficial bacteria that enhance plant growth, reduce abiotic stress, and protect against postharvest diseases, thereby supporting sustainable agriculture (Marian et al., 2025). Assessment of bacterial community dynamics is crucial for estimating endophytic community structure and its ecological behaviour, which are vital for understanding the molecular interactions with host plants (Abdullahi et al., 2020; Adhikary et al., 2021). Research on other mango cultivars by Adhikary et al. (2021) and Kumar et al. (2021a) reveals a shared microbiome that can inform our understanding of the Harumanis variety. According to Afzal et al. (2019), plant endophytic bacteria are microorganisms that live within plant tissues without harming their hosts. By developing symbiotic relationships with their host plants, these bacteria significantly improve plant production and health across a variety of environmental contexts (Kandel et al., 2017). They

live within plant tissues, form connections that promote nutrient exchange and enzymatic activities, and are referred to as plant growth-promoting endophytes (PGPE) (Kuźniar et al., 2024). The methods utilised by PGPE are like those of plant growth-promoting rhizobacteria (PGPR). Endophytic bacteria produce and release hormones that promote numerous physiological processes in plant tissues, thereby positively affecting plant development. They also produce a variety of bioactive compounds (such as alkaloids, amines, peptides, terpenoids and flavonoids) with a wide range of biological activities that can be carried out directly or indirectly, such as the solubilisation of mineral phosphate, potassium, and silicate (Biswas et al., 2023; Hassan et al., 2017), production of indole-3-acetic acid (Fouda et al., 2020), production of ammonia, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, nitrogen fixation, and biocontrol activities (Biswas et al., 2023).

The process of nitrogen fixation, which can involve certain endophytic bacteria, is a significant source of nitrogen for agricultural crops. In addition to reducing the need for artificial fertilisers, improving nutrient use efficiency, increasing crop yields, and reducing environmental pollution, endophytic nitrogen-fixing bacteria can be used as biofertilisers (Ng et al., 2024; Puri et al., 2020). Endophytes provide a variety of beneficial signals and metabolites that promote plant growth in various ways, which plants provide in exchange for protective niches (Ek-Ramos et al., 2019). Bacterial endophytes also mitigate the negative effects of abiotic stress such as salinity (Akbaba & Özden, 2023). Most plants harbour endophytes in their tissues; nevertheless, existing knowledge of PGPE and their biological activity does not align with the high prevalence of endophytes. Despite their importance in plant micro-ecosystems, the link between bacterial endophytes and their host plants remains poorly understood (Wang et al., 2024; Wu et al., 2021).

A better understanding of native plant endophytes may help reveal their potential to promote plant growth and support sustainable agricultural production. To date, no published study has examined bacterial endophytes associated with this cultivar. The discovery of PGP endophytic bacteria in this plant cultivar could be of biotechnological relevance in agriculture. Identifying the microbes naturally residing within the plant's tissues will allow the development of eco-friendly biocontrols, natural biofertilizers, and stress-tolerant crops that reduce reliance on synthetic chemicals. Therefore, the study aims to characterise endophytic bacterial diversity in the leaf tissues of Harumanis mango using 16S rRNA sequencing and investigate their potential to enhance plant growth and stress tolerance for sustainable agriculture.

MATERIALS AND METHODS

Sample Collection and Analysis

The leaves of the *M. indica* cv. Harumanis were collected from a Harumanis mango orchard plot in the Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia

Perlis (UniMAP), Sungai Chucuh Campus, 02100 Padang Besar, Perlis, Malaysia. The leaves were obtained through a randomised sampling method, which randomly selected healthy plants. From each of these plants, three mature leaf specimens that were healthy and asymptomatic, showing no prior damage or disease symptoms, were collected and pooled. The fresh leaf specimens were carefully placed in sterile, sealed bags, then placed inside polyethene boxes filled with ice and transported to the laboratory on the same day. The plant identification was tentatively recorded in the field. A total of four samples were used for the metagenomic analysis.

Surface Sterilisation

Surface sterilisation was performed on the collected leaves using the method described by Ruiz-Pérez & Zambrano (2017). The leaves were washed for 30 minutes under running water to remove any dirt, debris, and attached epiphytes. The leaves were then thoroughly cleaned with sterile distilled water after being washed with a detergent solution in tap water. Afterwards, the leaves were immersed in 90% (v/v) ethyl alcohol for 60 seconds. Then, immersed for 6 minutes in 5% sodium hypochlorite (NaCIO), followed by 30 seconds in 70% ethyl alcohol (v/v). Finally, the leaf samples were washed three times with sterile distilled water, and the final wash (200 µL) was pipetted and spread onto the nutrient agar (NA) plates. The leaves were dried using sterile filter paper before the efficiency of surface sterilisation was further tested by making an imprint on NA to ensure that every isolate comes from an endophytic rather than an epiphytic source and to examine the effectiveness of the procedure. A control plate containing NA media without a sample was used as a negative control. The plates were incubated at 28 ± 2.0 °C for 3 days and checked daily for microbial growth (Archana et al., 2020).

Genomic DNA Extraction and Sample QC

The genomic DNA from the Harumanis leaf sample was extracted using a commercial kit, the SEPA Plant DNA Isolation Reagent Kit, according to the manufacturer's instructions. The quality of the purified DNA was examined on 1% TAE agarose gel (Grobelak & Hiller, 2017; Nair et al., 2021). The concentration of DNA was measured using a spectrophotometer (Implen NanoPhotometer® N60/N50) and fluorometrically quantified with the iQuant™ Broad Range dsDNA Quantification Kit.

PCR Amplification, Library Preparation and Sequencing

The purified gDNA that passed DNA sample QC was amplified using locus-specific primer pairs. Bacterial 16S V3-V4 (5' to 3'): 16S V3-V4 Forward: CCTACGGGNGGCWGCAG; 16S V3-V4 Reverse: GACTACHVGGGTATCTAATCC. All the PCR reactions were carried out with REDiant 2X PCR Master Mix (1st BASE). Dual indices were attached to

the amplicon PCR using Illumina Nextera XT Index Kit v2 following the manufacturer's protocols. The quality of the libraries was measured using the Agilent Bioanalyzer 2100 System with the Agilent DNA 1000 Kit and fluorometric quantification by Helixyte GreenTM Quantifying Reagent. The libraries were normalised and pooled according to the protocol recommended by Illumina and then sequenced on the MiSeq platform with 300 PE. This was carried out by Apical Scientific Sdn. Bhd.

Bioinformatics Analysis

The raw sequencing data were quality-controlled using Trimmomatic to remove short and truncated reads. Subsequent downstream bioinformatics analysis was performed using the Mothur software package. Then, the near-identical clean reads were clustered together, and the chimaera reads were also removed. Subsequently, non-bacterial sequences such as 18S rRNA, chloroplast, and mitochondrial sequences were removed using Classify seqs and remove lineage options. Operational taxonomic units (OTUs) corresponding to the 16S rRNA sequences for each sample were determined using a 97% identity threshold for 16S gene variants at the species level. The taxonomy for each gene was analysed using RDP Classifier against the Silva database with a confidence threshold of 0.7. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was used to predict the functional potential of microbial communities in the leaf tissues from the 16S rRNA metagenomic data.

Data Deposition and Accession Number

The nucleotide sequences of the endophytic bacterial community from leaf tissues of *Mangifera indica* cv. Harumanis were submitted to the Sequence Read Archive (SRA) database of the National Centre for Biotechnology Information (NCBI) with a BioProject accession number PRJNA1353884.

RESULTS

Overview of 16S rRNA Amplicon Sequence, Richness and Diversity of the Endophytic Bacteria

A total of 126092, 107573, 115396, and 140617 clean reads were generated for samples 1, 2, 3, and 4, respectively. The clean reads were grouped into operational taxonomic units (OTUs) using a 97% similarity threshold. OTUs of each sample and the relative information of the tags were counted, and the most abundant OTU sequences in each cluster were selected as representative sequences for database blast and annotation. The number of OTUs per sample ranged from 100 to 162, with Sample 1 having the highest and Sample 3 the lowest, and a total of 593 OTUs were identified (Table 1). A Venn diagram represents the shared and unique bacterial taxa detected among the four different leaf samples obtained

from the metagenomic analysis (Figure 1). The numbers inside the overlapping regions indicate how many bacterial taxa are shared among the respective samples, while numbers found only within a single oval indicate unique taxa to that sample. Six (6) bacterial taxa are shared by all samples, forming the core bacterial community, indicating that certain bacterial groups are consistently associated with the plant's leaf surface regardless of minor differences in leaf location or sampling conditions. Rank abundance and rarefaction curves were generated and reached a plateau, indicating that the sample distribution and sampling depth were sufficient to determine the species richness in all the study areas, as shown in Figure 2.

Table 1
Sequence characteristics and the α-diversity of endophytic bacteria from the leaf tissues of M. indica *L. cv Harumanis*

Label	Group	Reads	OTU	Chao	Shannon
1	Sample-1	126092	166	9	5.050377
2	Sample-2	107573	165	13	4.781283
3	Sample-3	115396	100	9	4.558349
4	Sample-4	140617	162	9	4.978021

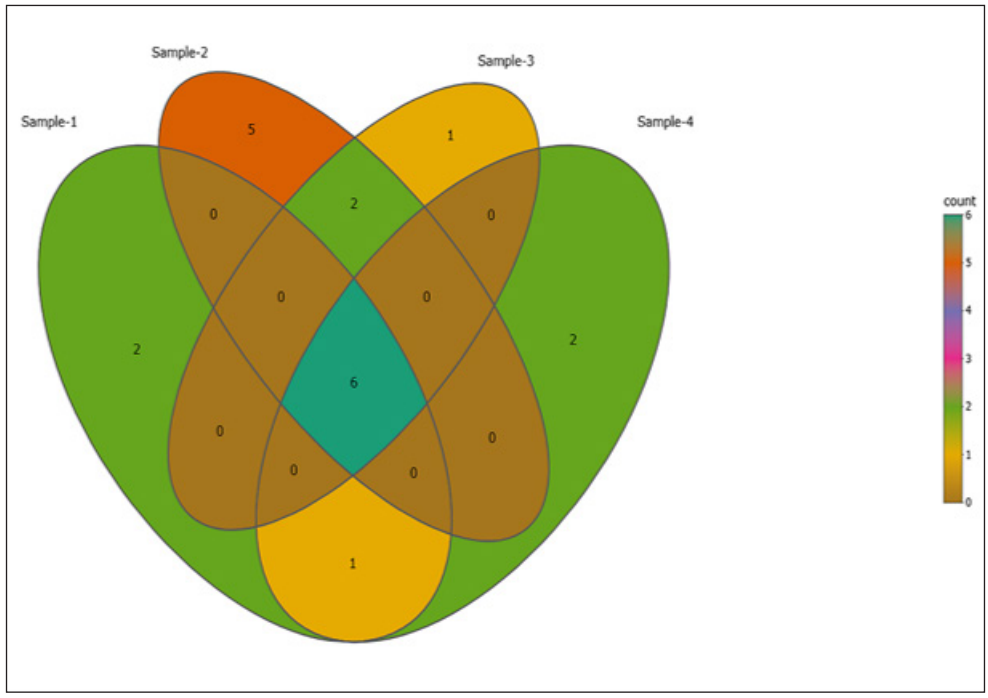


Figure 1. Shared and unique endophytic bacterial taxa detected among four leaf samples from the metagenomic analysis of *M. indica* L. cv. Harumanis

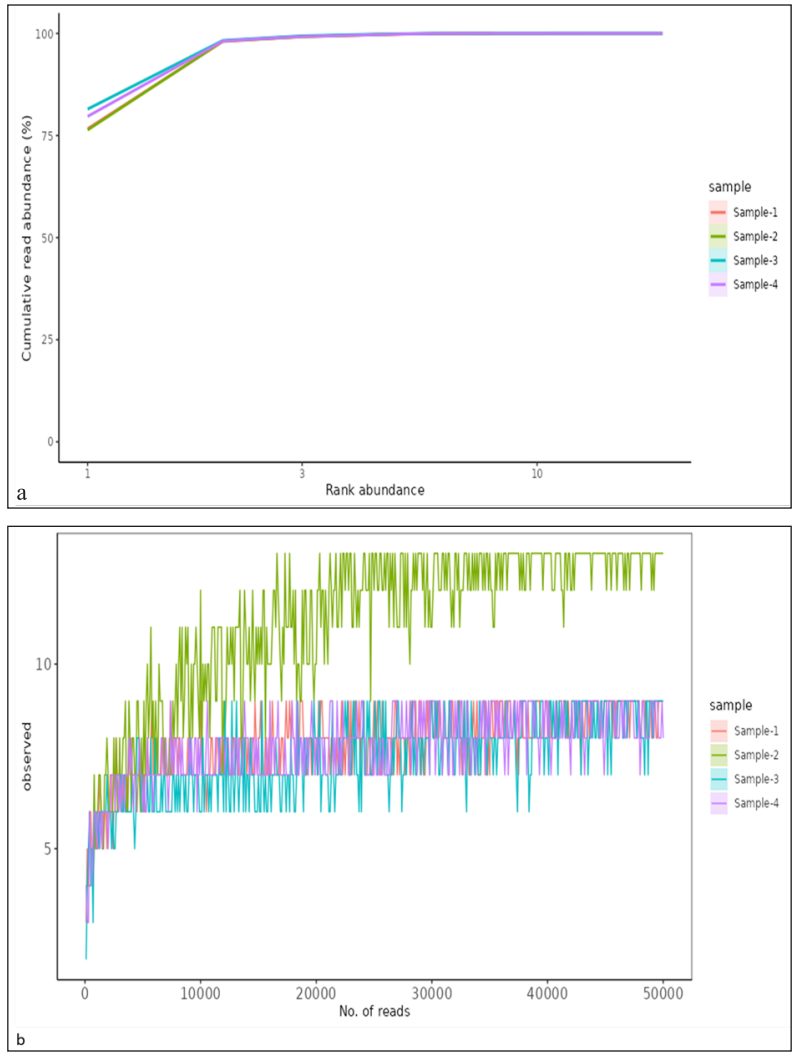


Figure 2. Rank abundance (a) and Rarefaction (b) curves generated from endophytic bacterial communities of *M. indica* cv. Harumanis leaves, indicating that the sample distributions and depth were adequate to assess species richness

The richness and diversity of the bacterial community in the leaves were estimated by computational analysis of alpha-diversity. Chao1 index estimated the number and richness of species. The Chao1 estimate for sample 2 was the highest (13), indicating greater species richness, while samples 1, 3, and 4, which had the lowest values (9 each), had lower species richness (Figure 3). A similar trend was observed in the Shannon diversity index values, with sample 2 again exhibiting the highest value. This indicates that it not only contains more species, but they are more evenly distributed. Sample 3 and Sample 4 have lower values, implying dominance by a few taxa (Figure 3).

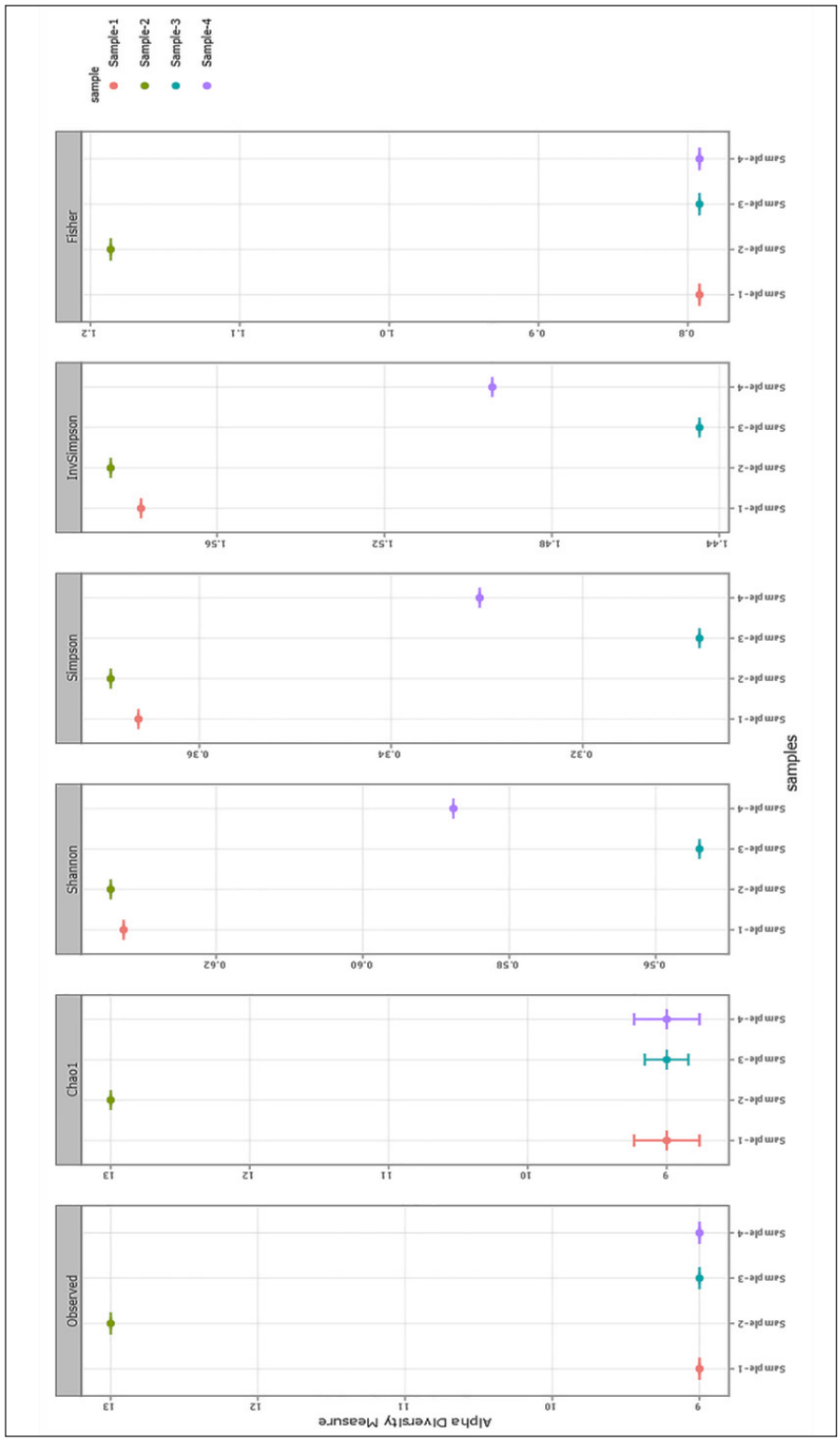


Figure 3. Alpha diversity indices of endophytic bacterial communities of leaves of *M. indica* cv. Harumanis. Sample 2 exhibited the highest bacterial richness and evenness, indicating greater community diversity and ecological stability compared to other samples

Structure of the Endophytic Bacterial Community in the Leaf Tissues of *Mangifera indica* cv. Harumanis

The most represented and dominant phylum among all the bacterial endophytes isolated from the leaf tissues of *Mangifera indica* cv. Harumanis was unclassified bacteria (51-82%), followed by Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%) (Figure 4). Sample 3 had the highest percentage of unclassified bacteria (82%), while Sample 2 had the highest percentages of Alphaproteobacteria and Gammaproteobacteria (15% and 8%, respectively). Actinobacteria were also found at the highest abundance (10%) in Sample 2. The top endophytic bacterial families detected in the leaves are presented in Figure 5. At the family level, the Bacteria-unclassified were also more abundant, with the highest occurrence in Sample 3 (82%). Caulobacteraceae, Sphingomonadaceae, Proteobacteria-unclassified, Bacterioles-unclassified and Enterobacteriaceae were much more abundant in Sample 2 (4% - 5%) than in other samples. The family Burkholderiales were also high in abundance in samples 4 and 1 (3% and 2%, respectively).

The composition of the bacterial community associated with the leaves of *M. indica* cv Harumanis revealed different taxa distributed and clustered across the samples (Figure 6). The relative abundance of each bacterial taxon in each sample is represented by the colour gradient, where red shades indicate high abundance and dominance, and blue shades indicate low abundance. The taxa labelled unknown mitochondria are the most abundant bacterial community across all samples, compared with other genera such as *Pseudonocardia, Caulobacter, Nocardioides, Achromobacter,* and *Ralstonia,* which show lower abundance. The samples and taxa are grouped into two hierarchical clusters based on similarity and patterns of bacterial abundance. On one cluster are samples 1 and 2, while on the other are samples 3 and 4. Samples in each cluster were closely grouped, indicating that they share a similar bacterial community. The two clusters are near but slightly apart, with minor variation likely due to physiological, environmental, or plant-age factors. The presence of unknown KD4-96 (family) in the samples indicates a bacterial community that could not be classified at the genus level. This further showed that the leaves are underexplored.

Endophytic Bacterial Functional Prediction

The predicted functional gene categories of the bacterial taxa in the leaf tissues by PICRUSt2 revealed diverse functions (Figure 7). Predicted functional genes are represented by corresponding colour intensity to indicate abundance, where yellow/red represent higher intensity or abundance of functional genes, and blue represents lower intensity or abundance. Hierarchical clustering by the tree-like structures (dendrograms) shows similarity among samples and among predicted functions. The samples were divided into

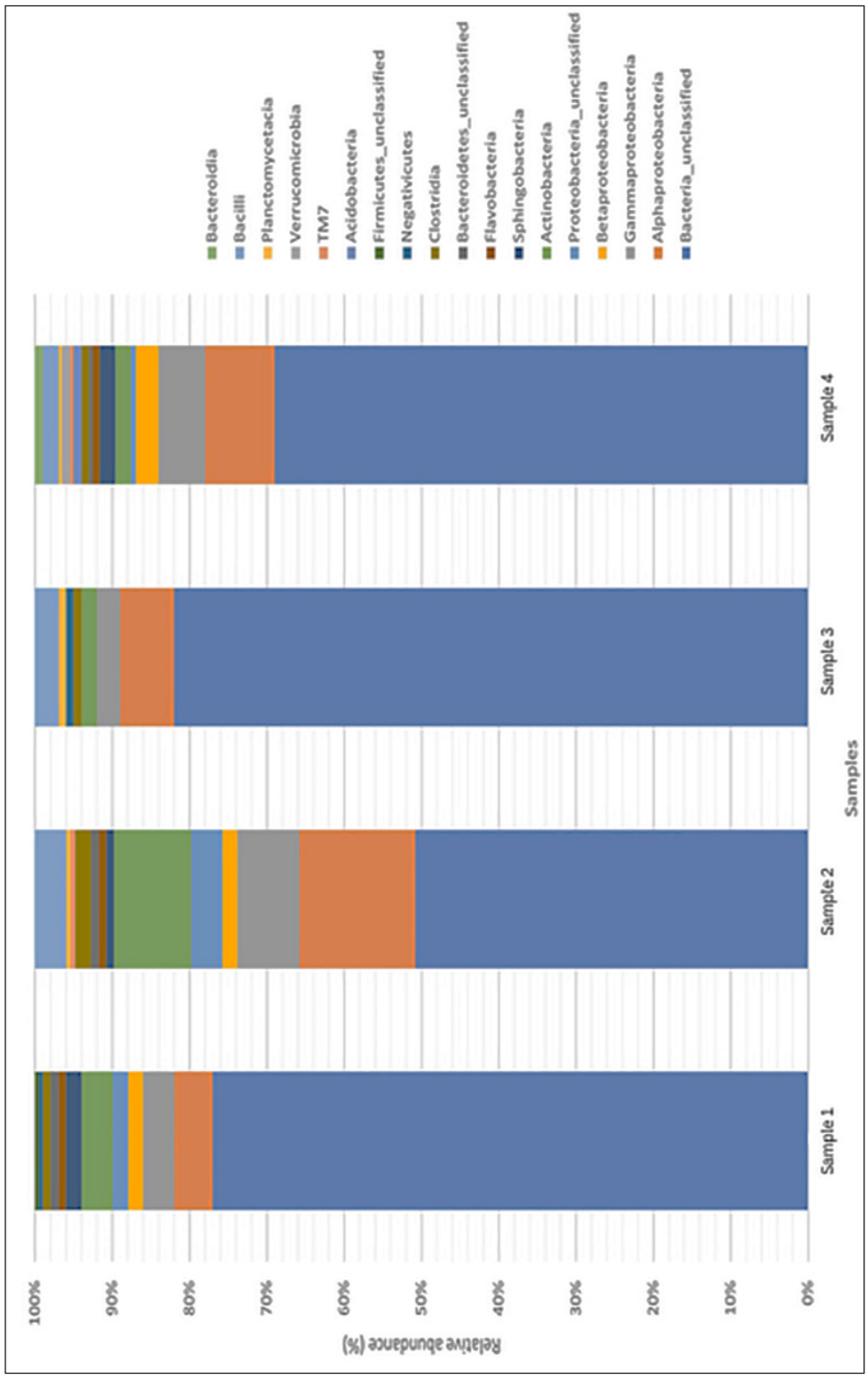


Figure 4. Relative abundance of endophytic bacteria at the phylum level identified from the leaf tissues of *M. indica* L. cv. Harumanis

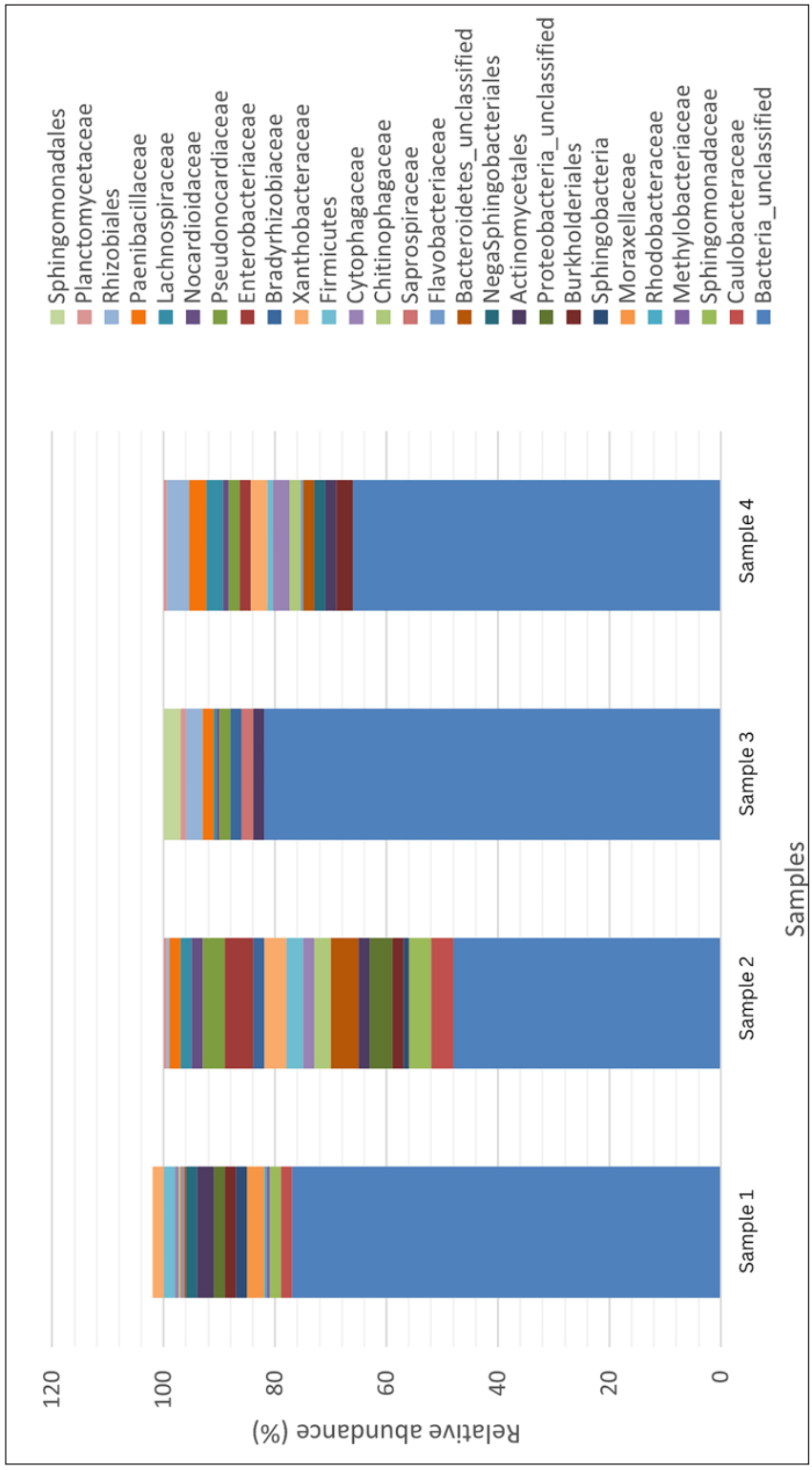


Figure 5. Relative abundance of endophytic bacteria at the family level identified from the leaf tissues of *M. indica* L. cv. Harumanis

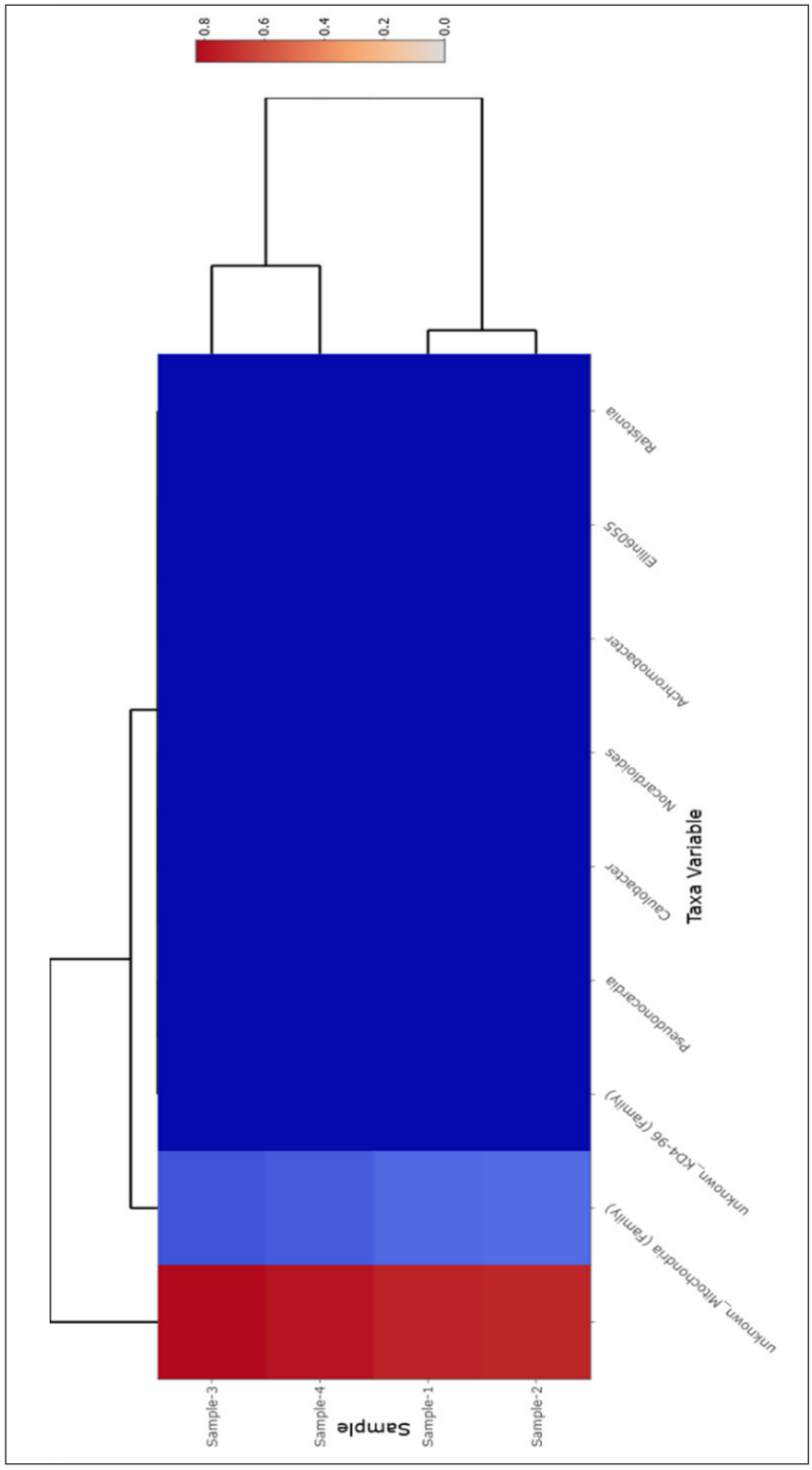


Figure 6. Overview of the taxonomic composition of endophytic bacterial genera associated with the leaf samples of *M. indica* L. cv Harumanis, showing how the bacterial genera are distributed and clustered across the samples

two major clusters: Sample 1, Sample 3, and Sample 4 in one cluster (indicating a similar functional profile), and Sample 2 in a separate cluster, suggesting a distinct functional composition of its bacterial community. The heatmap shows that Sample 2 has a broader region of high functional abundance (yellow/orange) compared to the others, indicating that its bacterial community possesses a greater number or diversity of metabolic genes. Furthermore, the distances between points by the ordination analyses of KEGG Orthologs (KOs) reflect how similar or dissimilar the samples are in their functional gene composition (Figure 8). For example, in CCA, Sample 1 (red) lies far apart, possibly under different environmental or host-associated conditions. NMDS and PCoA reveal functional divergence while maintaining relative distances (Samples 1 and 2 are closer in some plots, while 3 and 4 cluster more tightly). The distinct ordination positions suggest divergent metabolic potentials; e.g., carbohydrate metabolism, nitrogen cycling, or stress response genes may differ among samples. Samples 1 and 2 share more similar bacterial species, abundances, and functions, while Samples 3 and 4 contain more distinct bacterial profiles. This could reflect differences in leaf age, exposure, or microbial colonisation patterns.

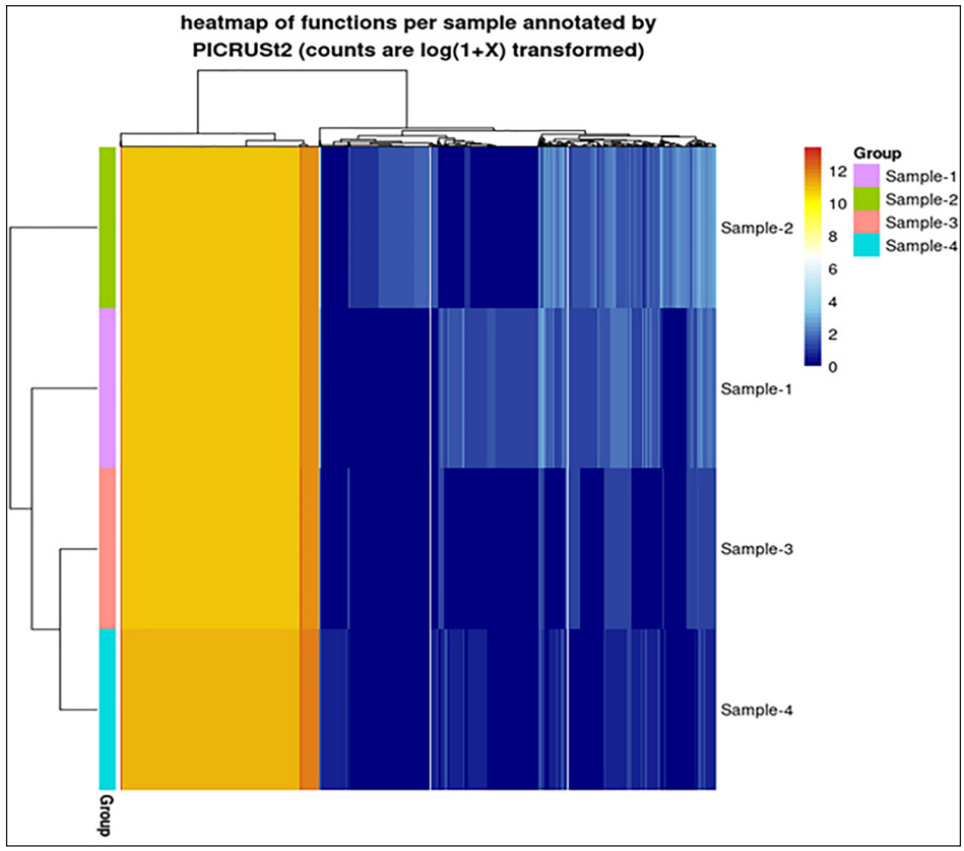


Figure 7. Functional heatmap of endophytic bacterial community in the leaf tissues of *M. indica* L. cv Harumanis predicted by PICRUSt2 based on 16S rRNA metagenomic gene data

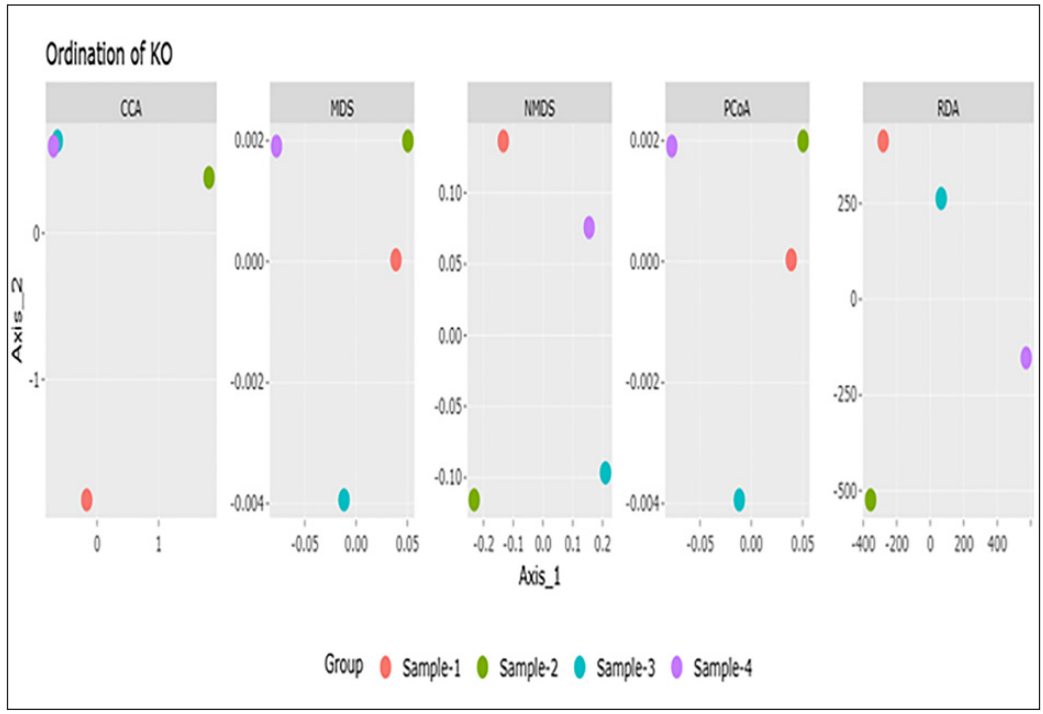


Figure 8. Ordination analyses of KEGG Orthologs (KOs) of endophytic bacterial community in the leaf tissues of *M. indica* L. cv Harumanis. Ordination plots (CCA, MDS, NMDS, PCoA, and RDA) show similarity or dissimilarity among samples based on their functional gene composition

DISCUSSION

In this study, 16S amplicon metagenomic analysis provided information on the composition, diversity, and distribution of endophytic bacteria associated with leaf tissue samples of harumanis mango. Since OTUs are thought to fairly represent community members, they are frequently used to infer diversity and functional features (Lladó Fernández et al., 2019). Six bacterial taxa are shared by all samples, forming the core bacterial community, indicating that certain bacterial groups are consistently associated with the plant's leaf surface regardless of minor differences in leaf location or sampling conditions. In general, any collection of microbial taxa, or the genomic and functional characteristics associated with those taxa, that are typical of a host or environment of interest is referred to as the core microbiome (Lladó Fernández et al., 2019; Neu et al., 2021). The occurrence of bacterial taxa in preferred locations, even when environmental factors strongly influence their distribution, clearly indicates that the bacteria are well adapted to the system (Abdullahi et al., 2021). Such core taxa are typically beneficial bacteria involved in various plant-beneficial functions, such as nutrient mobilisation, stress tolerance, and protection against pathogens (Finkel et al., 2017; Neu et al., 2021). The environment in Perlis is relatively hot

and dry, with distinct seasons directly shaping Harumanis mango's endophytic bacteria. Extreme temperatures, soil chemistry, and seasonal shifts determine which internal bacteria thrive, shaping the tree's defence mechanisms against diseases such as anthracnose and stem-end rot (Galsurker et al., 2020). The specific hot and dry conditions in northern Malaysia encourage the plant to produce higher levels of specialised secondary metabolites, which act as chemical filters and select for specific internal microbiota (Peron et al., 2025). Harsh, dry weather generally decreases overall microbial richness but increases the proportional abundance of resilient, stress-adapted bacterial communities inside the leaves and stems.

Higher community diversity and evenness in sample 2, as revealed by the computational analysis, could indicate a more stable and supportive environment in the leaf tissues. A more stable or nutrient-rich leaf environment that supports diverse bacteria could also indicate healthy phyllosphere conditions (Sivakumar et al., 2020). The alpha diversity metrics indicate that Sample 2 harbours the richest and most balanced bacterial community, while Samples 3 and 4 show reduced diversity, possibly reflecting environmental stress or host-specific selection. Environmental stress, leaf senescence, or antimicrobial compounds might have reduced bacterial colonisation (Bai et al., 2025). Even though leaves appear to be a harsh habitat due to their exposure to sunlight, dryness, and temperature fluctuations, many bacteria can live and even flourish there (Moitinho et al., 2020). These microbes may affect nutrient cycling, disease resistance, and plant growth (Niu et al., 2026). While some bacteria defend the plant from diseases, others generate hormones that promote development. These results highlight how leaf microenvironments strongly influence bacterial richness and evenness, shaping the structure and function of the leaf tissues' microbiome. Several variables, including plant species, leaf age, environmental factors, and agricultural techniques, affect the makeup of bacterial communities on leaves (Zhang et al., 2022). Gaining an understanding of these cultures will aid in developing environmentally friendly farming practices and increasing crop yields.

The most represented and dominant phyla among all the bacterial endophytes from the leaf tissues of *Mangifera indica* cv. Harumanis were bacteria-unclassified, Alphaproteobacteria, Gammaproteobacteria, and Actinobacteria. Other studies also reported similar results; for example, Li et al. (2025) showed that Proteobacteria were the dominant bacterial phylum in mango leaves. Adhikary et al. (2021), in their study exploring endophytic bacterial communities and their dynamics in the pre-flowering and post-flowering seasons in mango and its hemiparasites using 16S rRNA gene metagenomics, found that the most abundant genera belonged to the phyla Firmicutes, Proteobacteria, and Actinobacteria. Proteobacteria and Actinobacteria are key players when it comes to plant growth and health. Proteobacteria are involved in nutrient cycling, especially nitrogen fixation, while Actinobacteria are known for direct plant growth enhancement through the

production of hormones and indirectly through the production of antibiotics, solubilization of nutrients, and antagonistic activities against pathogens (Franco-Correa, 2016; Saberi Riseh, 2022). The higher percentages for unknown sequences at both the phylum and family levels is an indication that there are many potential new isolates to be discovered. The majority of Earth's microbial diversity remains uncharacterized in laboratory cultures, with many unidentified microorganisms, termed "microbial dark matter" (Lloyd et al., 2018), being numerically dominant across major environments. Additionally, about 25% of the microbial cell population belongs to phyla with no known relatives, suggesting these organisms are vital for ecosystem functions (Lloyd et al., 2018).

The study highlighted *Pseudonocardia, Caulobacter, Nocardioides, Achromobacter,* and *Ralstonia* as the most common genera associated with harumanis mango leaves. The genus *Pseudonocardia* is a member of the Pseudonocardiaceae family of actinomycetes. Research has shown that *Caulobacter* strains can increase plant biomass across a variety of plant species, aligning with agroecological relevance and biotechnological advancements (Berrios, 2022). Species of the genus produce secondary metabolites, some of which have antibacterial, antifungal, and antitumor properties, making them valuable in biotechnology (Riahi & Heidarieh, 2022). A study by Ghodhbane-Gtari et al. (2019) revealed two plant-growth-promoting actinobacteria of the genus *Nocardia*, strains BMG51109 and BMG111209, to induce root nodule formation in *Casuarina glauca.* Species of *Achromobacter,* such as *Achromobacter piechaudii,* are known to produce ACC deaminase, which has been shown to alleviate drought stress in plants by increasing biomass and reducing ethylene levels (Yang et al., 2009). The unknown genus (unknown KD4-96) present in the uncultured bacterial lineage may represent a novel taxon in the inner leaf tissues of the harumanis mango. It also highlights the potential of metagenomic studies to reveal unexplored microbial diversity that traditional culture-based approaches couldn't. Deciphering the plant microbiome through next-generation sequencing of the 16S rRNA is a good method to identify phyla but not very suitable to classify species or genus (Sharma et al., 2020). The leaf tissues serve as a home to many uncultivated microorganisms (Moitinho et al., 2020). Also, because of interspecies molecular dependence, it is essential to comprehend the molecular underpinnings of the social networks in this habitat to obtain fresh perspectives on the ecology of the enigmatic community members (Moitinho et al., 2020).

The predicted functional gene categories of the bacterial taxa in the leaf tissues were divided into two major clusters, with Sample 1, Sample 3, and Sample 4 in one cluster (indicating a sharing of similar functional profiles) and Sample 2, on the other hand, in a separate cluster, suggesting a distinct functional composition of its bacterial community. The relationship between real taxa and molecular taxa appears to be far more nuanced. Only a few genes (the core genome) are usually shared among strains of the same bacterial

species (preferably belonging to the same OTU), other genes are strain-specific and distinct (Lladó Fernández et al., 2019). Even though leaves appear to be a tough habitat due to their exposure to sunshine, dryness, temperature fluctuations, UV radiation, and nutrient availability, many bacteria may live and even flourish there. These microbes may have an impact on nutrient cycles, disease resistance, and plant growth (Dastogeer et al., 2020; Sivakumar et al., 2020). Various environmental factors and conditions may account for variation among samples, including sunlight, humidity, temperature, age, and nutrient availability, which are known to affect microbial gene functions. The microbial community with increased metabolic activity, possibly involved in the food cycle, secondary metabolite generation, or stress tolerance, may be reflected in Sample 2 with enrichment in metabolic functions. This may indicate that a metabolically active microbiome is supported by the physicochemical conditions of Sample 2 (younger leaf, increased nutrient exudation, or more moisture). The key microbial processes necessary for plant-microbe interactions are likely represented by the core functions shared among the other samples, such as nitrogen metabolism, stress resistance and antioxidant mechanisms, phytohormone biosynthesis (auxins, cytokinins), plant exudate degradation, and carbon utilisation. Numerous significant anticipated biosynthetic gene clusters for plant phytohormones, secondary metabolites, and novel natural products were found in the genome of Nocardia (Ghodhbane-Gtari et al., 2019). The distinct ordination positions suggest divergent metabolic potentials; for example, carbohydrate metabolism, nitrogen cycling, or stress response genes may differ among samples.

CONCLUSION

The leaves of *Mangifera indica* cv. Harumanis host a diverse community of beneficial endophytic bacteria, primarily belonging to the phyla Alphaproteobacteria (5-15%), Gammaproteobacteria (3-8%), and Actinobacteria (2-10%). Dominant genera revealed in the leaf tissues included *Pseudonocardia, Caulobacter, Nocardioides,* and *Achromobacter,* which are generally considered beneficial in environmental and agricultural contexts. These bacteria possess several plant growth-promoting traits such as nutrient cycling, defense against pathogens and stress tolerance, indicating their potential role in sustainable agriculture. Additional research may provide a more thorough understanding of the functional genes and interactions between the bacteria and the Harumanis mango. By utilising these natural microbial communities, it may be possible to promote healthier plants and lessen reliance on artificial fertilisers.

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Reviving Peatland Sustainability: Insights of Perception, Willingness to Invest, and Financial Feasibility of Agroforestry Models

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ABSTRACT

Several agroforestry-based peatland restoration models are currently being implemented. However, comprehensive evidence analysing farmers' perceptions, willingness to invest (WTI), and cost-benefit insights remains limited. Addressing this gap is essential for optimising sustainable peatland restoration. Therefore, this study aimed to analyse perceptions of peatland and agroforestry models, examine the factors determining farmers' WTI, and calculate the costs and benefits of agroforestry systems on peatlands. The study was conducted in Perigi Village, Pangkalan Lampam District, and Perigi Village, Kayu Agung District, Ogan Komering Ilir Regency, South Sumatra, Indonesia, where farmers had practised agroforestry for more than five years. Data were collected through a survey of 85 respondents and analysed using tabulation analysis, logit model, and cost-benefit analysis (IRR, NPV, Net B/C, and Gross B/C). The results showed that the majority of respondents perceived agroforestry schemes as an effective method for enhancing productivity by combining various commodities, including agrosilviculture, agrosilvofishery, and agrosilvopastoral systems. These systems were also considered capable of increasing farmer incomes and reducing peatland degradation. Approximately 72% of smallholder farmers in Indonesia were willing to implement the agroforestry model. Factors such as indigenous status, education, production planning, and farmer group activity positively influenced WTI, while dominant land use patterns and knowledge of agroforestry had negative effects. The optimal implementation of agrosilviculture systems, compared with agrosilvofishery and agrosilvopastoral, was necessary for sustainable development. This study suggests promoting smallholder farmers' adoption of agroforestry practices through

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improved decision-making support, social proximity, technical information, welfare benefits, and addressing productivity constraints.

Keywords: Agrosilvopastural, agrosilviculture, agrosilvofishery, restoration, welfare

INTRODUCTION

Tropical peatlands are essential as global carbon repositories. Although discovered in temperate, tropical, and boreal zones, these peatlands store the highest amount of carbon per unit area due to the thickness of the layers and abundance of above-ground biomass (Austin et al., 2025). Furthermore, tropical peatlands offer a range of ecosystem services, including the provision of timber and non-timber forest products, wildlife habitats, and water storage. They play a crucial role in regulating the water balance by mitigating the effects of floods and droughts through water retention following precipitation events and gradual release during dry periods (Loisel & Gallego-Sala, 2022; Pudyatmoko et al., 2023). The area of peatland owned by Indonesia is the second largest in the world after Brazil. It is estimated to cover half of all peatland areas in the entire tropical region and account for approximately 84% in Southeast Asia (Jessup et al., 2020; Lestari et al., 2024). The area in Indonesia is approximately 14.91 million ha, spread across Sumatra 6.44 million ha (43%), Kalimantan 4.78 million ha (32%), and Papua 3.69 million ha (25%) (Wicaksono & Zainal, 2022; Yuwati et al., 2021).

South Sumatra Province has an area of 1.28 million hectares of peatland, in which 0.78 million hectares are degraded and covered with grass and shrubs. This substantially increases the risk of peatland fires, particularly during periods of extreme drought, underscoring the need for integrated and sustainable ecosystem management. The issue continues to reoccur and has become a very serious challenge in the southern part of Sumatra Province. The peatlands of Indonesia, particularly in South Sumatra, are crucial ecosystems with large carbon stocks and unique biodiversity. However, these areas face significant threats from deforestation, unsustainable agricultural practices, and recurring fires, leading to degradation and loss of ecological function (Lestari et al., 2021; Wildayana et al., 2018; Winarno et al., 2022).

The direct impact of forest and peatland fires on humans is the loss of livelihoods, particularly among communities that depend on natural resources for farming, livestock, hunting, fishing, and other subsistence activities. The fires destroy plantations, agricultural lands, and crops, thereby reducing agricultural productivity. In addition to the economic impact, the global environment is also being affected. The combustion of vegetation and peat releases large amounts of carbon into the atmosphere, intensifying global warming. Recurrent burning also depletes soil nutrients and degrades peatland quality, diminishing its ecological functions and economic value for sustainable human use (Wildayana et al., 2018; Wildayana & Armanto, 2018).

The preservation of surviving peatlands is crucial for achieving effective and sustainable restoration. Based on observation, the restoration initiatives are particularly important for generating economic benefits that help secure local revenue (Gunawan et al., 2021; Purnomo et al., 2024). Restoration activities can be implemented through peatland cultivation methods based on the agroforestry concept that correlates with the needs for community welfare. Agroforestry provides a sustainable livelihood alternative that enhances biodiversity and ecosystem services while reducing pressure on forests by enabling the production of forest products within agricultural landscapes (Sheppard et al., 2020; Qurniati et al., 2024). Given these benefits, the Indonesian Ministry of Environment and Forestry has recommended agroforestry as the best practice for peatland restoration. Traditional agroforestry practices on peatlands are often limited to cultivation along riverbanks, where communities manage water levels through tidal movements and canal blockages. Various regions in the area also adopt polyculture practices, such as the surjan system, which combine water-based plants with plants or trees requiring drier soil (Rozaki et al., 2021; Triwanto et al., 2023; Wijayanto et al., 2022).

Although widely recognised as a sustainable method for peatland restoration, agroforestry adoption among smallholder farmers remains limited (Gori Maia et al., 2021; Rozaki et al., 2021; Triwanto et al., 2023). This condition shows that farmers' decisions are influenced not only by economic feasibility but also by socio-economic perceptions and investment considerations (Pusvita et al., 2024; Wijayanto et al., 2022). Previous studies showed that agroforestry provides both ecological and economic benefits. However, the factors influencing willingness to invest (WTI) in peatland agroforestry systems remain insufficiently explored (Adriani et al., 2024; Ema et al., 2023).

Farmers' perceptions of the agroforestry model will determine the WTI in the implementation. Several studies on the agroforestry model have focused more on willingness to pay (WTP) than WTI, even though these are fundamentally different concepts. WTP is the maximum amount of money an individual is disposed to pay towards obtaining a particular good, service, or benefit, or to avoid a negative impact. This concept can be used for environmental economic assessment, ecosystem service valuation, public policy, and social feasibility studies (Gosh et al., 2022). Meanwhile, WTI is the amount of money or resources an individual or investor is committed to investing in a project or innovation, with the expectation of long-term benefits or impact. This concept is used for general applications of green investment, sustainable agriculture projects, bioenergy, and technological innovation (Karasmanaki et al., 2019; Romero-Castro et al., 2023). The implementation of agroforestry on the peatlands is not only a technical process. Understanding farmers' perceptions and motivations, as well as the factors underlying investments in agroforestry, is equally essential.

Previous studies have given limited attention to WTI, emphasising the need to identify its key determinants. Existing agroforestry literature has predominantly examined WTP,

while WTI remains unexplored. Although WTP reflects the level of individual preferences for selected benefits or environmental improvements, WTI captures a longer-term commitment to resource allocation and investment (Karasmanaki et al., 2019; Romero-Castro et al., 2023). Therefore, understanding farmers' perceptions and investment intentions is essential for promoting the successful implementation of agroforestry systems in peatlands.

Based on these considerations, this study aims to: (1) analyse farmers' perceptions of agroforestry implementation in peatland areas, (2) identify the factors influencing WTI, and (3) evaluate the economic feasibility of agroforestry models in the peatlands of South Sumatra. The result is expected to address the existing gap regarding the limited understanding of WTI determinants and the economic value of agroforestry as a sustainable restoration strategy.

METHODOLOGY

Selection of Study Area

The greatest amounts of degraded peatlands in the region were discovered in Ogan Komering Ilir (OKI) Regency, a part of South Sumatra Province, Indonesia. The degraded peatlands cover 1,687,348 hectares, or 49.25% of the province's total area. Furthermore, the study was conducted in (1) Perigi Village, Pangkalan Lampam District, and (2) Perigi Village, Kayu Agung District, Ogan Komering Ilir Regency, from June 2024 until completion. These locations were selected purposively, considering that a Pilot Project for the implementation of integrated peat restoration trials through the agroforestry model had been conducted since 2017 (Figure 1). The peatland in both villages was classified as degraded and characterised by a decline in ecological, production, and hydrological functions. The impact of this degradation is the decline in land productivity, compounded by frequent yearly burning.

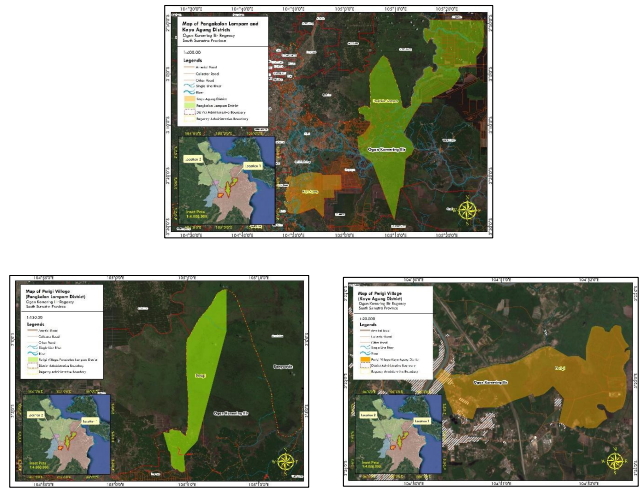


Figure 1. Study site

Survey Design and Data

A survey method was used to obtain quantitative data on farmers' beliefs, opinions, characteristics, behaviours, and perceptions regarding peatland restoration and agroforestry practices. The method was selected because it captured field-based conditions while providing an appropriate basis for examining the relationships among variables and testing the proposed hypotheses.

The present study adopted a balanced stratified random sampling method to obtain a representative sample of farmers participating in the peatland restoration program across the selected villages. The target population was stratified by village, and respondents were randomly selected from each stratum in proportion to its population size. This method improved sample representativeness and reduced potential bias related to population heterogeneity, consistent with established sampling principles. (Choi et al., 2024; Howell-Moroney, 2023). A sample proportion of 30% from each group was considered sufficient to represent the overall population because the characteristics of farmers within each stratum were relatively homogeneous. Furthermore, the selected proportion was considered adequate to capture respondent variability and support reliable statistical analysis of peatland restoration activities (Goswami et al., 2024; Howell-Moroney, 2023).

A quantitative survey method was used to obtain data that occurred in the past or present, about beliefs, opinions, characteristics, behaviour, relationships of variables, as well as to test certain hypotheses. This study adopted a balanced stratified random sampling method for farmers who had participated in the peatland restoration program, as detailed in Table 1.

Table 1
Sampling method

No.	Location	Population	Sample	Percentage
1	Perigi Village, Pangkalan Lampam District, Ogan Komering Ilir Regency	167	50	30%
2	Perigi Village, Kayu Agung District, Ogan Komering Ilir Regency	120	36	30%

The questionnaire consisted of several sections, including (1) general characteristics of the respondents, (2) perceptions of peatlands and agroforestry models, (3) WTI in agroforestry models, and (4) the costs and benefits associated with selected models. The primary study question was: To what extent are farmers who have received assistance through agroforestry programs willing to invest independently to sustain the implementation of various agroforestry models? The description of the variables is presented in Table 2.

Table 2
Description of study variable

Name of Variable	Variable	Variable Definition	Measurement
X1	Age		Years
X2	Indigenous people		1 = Yes: 0 = No
X3	Gender		1 = Man; 0 = Woman
X4	Education		Years
X5	Family size		Person
X6	Land productivity	Land is used or not used	Land is used =1 Land is not used = 0
X7	Land scale	Land area	Ha
X8	Land certificate		1 = Own ownership; 0 = Non-ownership
X9	Dominant land use for agriculture	Rice, corn, and others	1 = Yes; 0 = No
X10	Dominant land use for plantation	Rubber, Palm Oil, and others	1 = Yes; 0 = No
X11	Plans to increase productivity	Having a plan to improve land productivity	1 = Yes; 0 = No
X12	Have knowledge of the technical aspects of restoration	Level of knowledge and skill about agroforestry	1 = High; 0 = Low
X13	Member of farmers organisation	Active Member of farmers community	1 = Active; 0 = Passive

A logistic regression model was used to address the second objective, namely analyzing the WTI (Karasmanaki et al., 2019). This method was selected for the ability to accommodate non-linear relationships between the dependent (WTI) and a range of independent variables, both categorical and continuous. Through this model, it became possible to identify demographic, social, and farm-related characteristics that had predictive power over farmers' propensity to adopt innovative commodity combination models. Respondents who were willing and unwilling to invest in agroforestry were coded as 1 and 0, respectively. The binary nature of the dependent variable necessitated the use of a logistic regression model to analyse the effects of independent variables on the respondents' WTI in agroforestry. The model specification is given in Equation 1.

$$Z_i = ln\left(\frac{p}{1-p}\right)$$
$$= \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 \quad [1]$$
$$+\beta_7 x_7 + \beta_8 x_8 + \beta_9 x_9 + \beta_{10}$$
$$x_{10} + \beta_{11} x_{11} + \beta_{12} x_{12} + \beta_{13} x_{13} + \varepsilon \ldots$$

In the estimated model, α is the intercept and βi is the regression coefficient that provides the expected change in WTI when the corresponding independent variable increases by one unit (ceteris paribus).

The third objective of this study was achieved by a cost-benefit analysis, which evaluated the total economic value of the proposed investment. The profitability analysis was generally categorised into four parts, namely Financial analysis with indicators of Net Present Value (NPV), Benefit Cost Ratio (BCR), Internal Rate of Return (IRR) and Payback Period (PP). An extended cost-benefit analysis method was applied to evaluate the benefits of selected agroforestry scenarios on degraded peatlands due to restoration efforts.

To assess the most suitable agroforestry method for sustainable peatland utilisation, this section presents a comparative overview of three integrated land-use models, namely Agrosilviculture, Agrosilvofishery, and Agrosilvopastural. Each model integrated forestry components with agricultural, aquaculture, or livestock elements within a one-hectare plot, reflecting a multifunctional land management strategy. The comparison covered critical dimensions such as economic life, discount rate, types and quantities of commodities planted or reared, spatial arrangement (planting distance or infrastructure), as well as projected final outputs. The Agrosilviculture model combined *meranti* timber production with pineapple and rice farming over a relatively short economic life of 10 years. In contrast, the Agrosilvofishery model, with a longer horizon of 30 years, integrated *jelutung* trees, pineapples, and catfish, demanding higher initial investment and more complex water infrastructure. The Agrosilvopastural system, extending over 35 years, integrated *nyamplung* trees with rice farming and duck husbandry, showcasing diversified biological outputs and intensive management needs. This structured comparison enabled stakeholders, particularly farmers, policymakers, and development agencies, to identify trade-offs and select the most context-appropriate agroforestry model based on ecological compatibility, economic returns, and operational feasibility. To further support decision-making, the comparative framework also emphasised the varying levels of technical capacity and institutional support required for each model's implementation. Additionally, differences in output timing, whether short-term annual yields or long-term forestry benefits, played a crucial role in determining model attractiveness to smallholder versus commercial-scale actors.

A comparative analysis of the agrosilviculture, agrosilvofishery, and agrosilvopastural systems showed distinct configurations of integrated land-use practices (Chappa et al., 2024), each with its own set of ecological, economic, and managerial implications. These agroforestry models have been specifically designed to optimise the sustainable utilisation of peatland resources by combining forestry with agricultural, aquacultural, or livestock components (Zerbe, 2022). The agrosilviculture model presents the most

accessible entry point among the three, primarily due to relative simplicity, shorter economic time horizon, and lower initial capital requirements (Mössinger et al., 2022). It integrates timber-yielding tree species with seasonal crops and horticultural commodities, thereby enabling farmers to generate periodic income from annual harvests while awaiting returns from the longer-term forestry component. The model is particularly appealing to smallholder farmers or community-based enterprises with limited investment capacity. This is because agrosilviculture allows for the gradual accumulation of revenue and mitigates the financial risks commonly associated with long- maturity agroforestry ventures (Dhakal et al., 2022).

The agrosilvofishery model represented a more complex and technically demanding system, designed to exploit the hydrological characteristics of peatlands by incorporating aquaculture alongside forestry and agricultural crops (Indrajaya et al., 2022). The model fostered ecological and economic diversification by enabling concurrent outputs from tree-based products, aquaculture yields, and fruit harvests. However, its implementation required higher levels of technical knowledge, greater financial inputs, and infrastructural development, such as fish pond construction and water management systems (Cheyo et al., 2024).

The agrosilvopastural model was the most integrated and multifunctional method among the three. It combined tree cultivation, seasonal cropping, and livestock rearing into a single land-use system, thereby facilitating nutrient cycling, biodiversity conservation, and multi-product outputs (Mantziaris et al., 2022). The inclusion of livestock, such as ducks, not only diversified income streams but also contributed to improved farm resilience and ecological synergies (Chappa et al., 2024). However, the model demands high levels of managerial skill, continuous labour input, and infrastructural readiness, particularly for animal housing and health management. Its extended project horizon and complex operations suggested that the model is best suited for organised farming collectives or individual farmers with long-term investment capacities and technical competencies.

The selection of an appropriate agroforestry model should be conducted in consideration of a confluence of factors. These include the ecological characteristics of the site, the socio-economic profile of the farming community, the availability of institutional support, and the temporal trade-offs between short-term returns and long-term sustainability. While the agrosilviculture model offered immediate benefits with lower complexity, the agrosilvofishery and agrosilvopastural systems provided broader ecological and economic advantages. These systems required greater investment, labour, and technical expertise. The comparative strengths and constraints of the models emphasise the importance of context-specific planning in the sustainable development of peatland-based agroforestry systems.

RESULTS AND DISCUSSION

Farmers Characteristics

The results showed that the majority of peatland farmers in South Sumatra were male. Approximately 66 and 34% of respondents were male and female farmers, as detailed in Table 3. Both genders play important and different roles in supporting household livelihood strategies. However, males tended to play a more visible role in meeting the household's economic needs (Yazid et al., 2024). In terms of age, the older adults dominated the respondent profile. This group constituted approximately 72% of the total respondents, ranging from 38 to 70 years of age. The trend points to the low participation of younger generations in agricultural activities and shows a continuous challenge in the regeneration of the agricultural workforce. The low participation of young people may be attributed to limited economic prospects in farming, the attraction of off-farm employment, and the perception that peatland farming offered uncertain long-term prospects (Adriani & Septiani, 2023). Most farming households had five to seven members in terms of size. This was higher than the result reported for households in Java. More family members are proportional to higher economic pressure, which encouraged farmers to maximise the productivity of their peatland in order to meet needs. The education level of respondents was generally low, with 78% only receiving primary education. Limited educational background among farmers may constrain the ability to accept new knowledge and perspectives as well as slow down the adoption of agricultural technologies and innovations (Rosnan & Yusof, 2023). Although training and agricultural extension programs are available, their effectiveness in promoting innovation adoption may be constrained by the generally low educational attainment of farmers. However, the participation rate in these programs was relatively high. A total of 58% of the respondents had attended training sessions or extension activities organised by government agencies or academic institutions as part of community empowerment initiatives. In addition to developing technical expertise, the informal learning opportunities were expected to encourage better cooperation among farmers and improve the implementation of future agricultural development programs.

In this study, 90% of the respondents cultivated legally and self-owned peatland. Meanwhile, the remaining 10% cultivated land that belonged to other family members or leased assets from their parents. The size of the average peatland owned by farmers was 1 to 2 hectares, and only 2% of the respondents had 5-to 6-hectare holdings. This distribution showed that land ownership for peatland farmers tends to be restricted. These constraints can limit the scope for extending farming operations and increasing profitability but can be managed to some extent by agricultural intensification. Most respondents had also been long-term experienced peatland farmers. Approximately 33% had been farming for between 2 and 10 years, while the other 67% had more than ten years of experience.

This accumulated knowledge is possibly to have a positive influence on the perception and decision of farmers to manage their farming activities.

The household income distribution showed that the majority of the respondents fell within the low-income category, while relatively few fell within the middle-income category, and only a small proportion belonged to the high- and very high-income categories. The trend is consistent with the predominance of smallholder farmers whose monthly income was typically low and often fluctuated over time. These financial conditions reflected the economic vulnerability of the farming households that remained heavily reliant on seasonal agricultural production for income. The households had a limited ability to absorb the production costs and meet expenses during the non-harvest periods.

Table 3
Farmers characteristics

Variable	Percentage (%)	Average	Standard Deviation (±)
Gender			
Man	66	-	-
Woman	34		
Age			
27-37 years	28		
38-48 years	28	46	9.3 (70.27)
49-59 years	36		
60-70 years	8		
Family Members			
2-4 persons	24	4	1.3 (7.20)
5-7 persons	76		
Formal Education			
No School and Primary School	78		
Junior High School	12	8	2.4 (16.60)
Senior High School	4		
Bachelor Degree	6		
Land Status			
Owned by Individuals	90	-	-
Leased Land	10		
Land Scale			
1-2 ha	72	2.00	1.41 (61.00)
3-4 ha	26		
5-6 ha	2		
Farm Experience			
2-10 years	32		
11-19 years	22		
20-28 years	20	20	11.8 (50.21)
29-37 years	18		
38-46 years	6		
> 47 years	2		

Table 3 (*continued*)

Variable	Percentage (%)	Average	Standard Deviation (±)
Income Range (IDR/Month)			
1,000,000 - 4,500,000 (Low)	47.67		
> 4,500.000 - 8,000,000 (Medium)	36.05	5,258,140	3,301,608 (62.78)
> 8,000,000 -11,500,000 (High)	11.63		
> 11,500,000 - 15,000,0000 (Very High)	4.65		

Farmers' Perception of the Agroforestry Model for Optimising the Sustainability of Agricultural Businesses on Peatlands

Farmers' perceptions can be positive or negative depending on the assessment of their experiences and the conditions of peatland management. Figure 2 provides a more detailed overview of these perceptions, including farmers' opinions on the advantages and difficulties of peatland utilisation. The results provided an important empirical basis for the design of more adaptive, participatory and context-specific intervention strategies, thereby favouring a transition towards more sustainable peatland-based agricultural systems. Farmers' knowledge of agroforestry remained relatively limited, as confirmed by 58.1% of respondents (50 out of 86 respondents). Although farmers were familiar with peatland utilisation, their understanding of agroforestry and paludiculture systems remained relatively low. The limited knowledge may be associated with inadequate access to extension services, technical training, and practical exposure to sustainable peatland management practices.

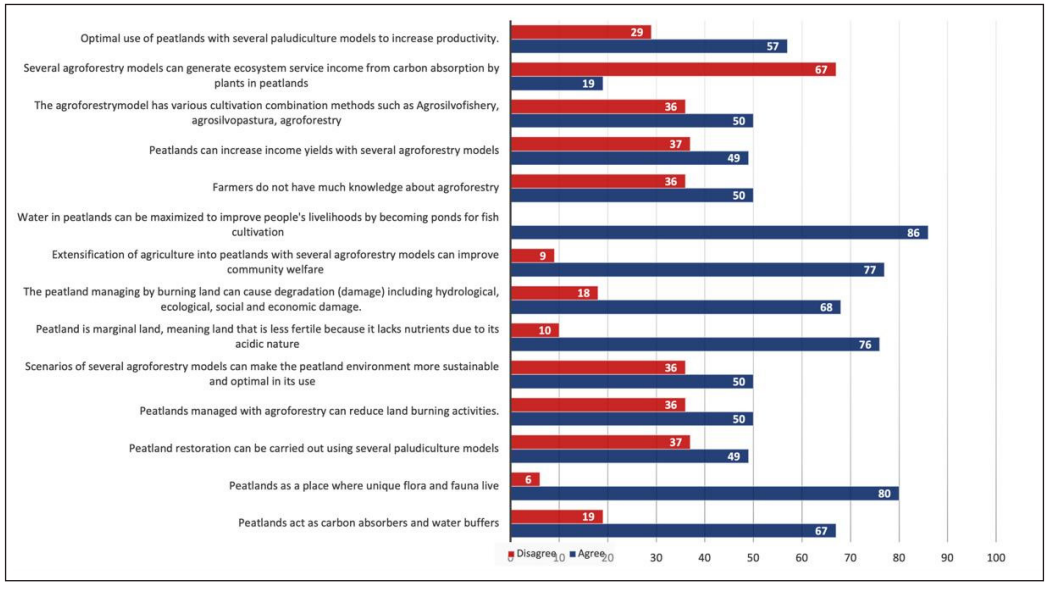


Figure 2. Farmers' perception of the agroforestry model for optimising the sustainability of agricultural businesses on peatlands

Optimal Utilisation of Peatland with the Agroforestry Model

The majority of respondents agreed that optimal utilisation of peatland through various alternative commodity combination models can enhance productivity. The agroforestry commodity integration model combines multiple commodities, such as agricultural crops and aquaculture in wetland areas, to increase farmers' incomes while promoting environmental sustainability (Qurniati et al., 2024). This was further supported by a study where combining agricultural crops with native trees or other plants provided additional economic value and mitigated the risk of peatland degradation (Applegate et al., 2022).

Agroforestry Model as a Multifunctional Method

Respondents expressed a high level of consensus on the role of the innovative commodity combination model in promoting diverse cultivation methods, including agrosilvofishery, for income diversification. Agrosilvofishery integrates food crops, timber trees, and aquaculture activities within a single land-use system, thereby creating opportunities for income diversification among farmers in line with (Adriani et al., 2024; Goswami et al., 2024). This method not only contributed to enhancing household food security but also served as an adaptive strategy to address economic and environmental risks, while simultaneously providing access to more sustainable alternative income sources. Numerous studies have shown that this type of diversification can also improve environmental sustainability by preventing the overexploitation of land resources (Agbodjato & Babalola, 2024; Mondal & Palit, 2022).

Peatlands as a Source of Increased Income and Alternative Cultivation

More than 50% of the respondents agreed that the peatlands offered great potential as an alternative area of cultivation to increase the income of the farmers. The respondents further stated that integrated commodity systems, particularly paludiculture, generated a variety of high-value products from peatlands, including sap, honey and freshwater fish. According to previous studies, multi-commodity farming systems tended to generate more stable and sustainable income than monoculture systems, which were generally more susceptible to market and climatic variability (Adriani et al., 2024). This strategy was very important to improve farmer welfare while maintaining the sustainability of the ecosystem (Syahza et al., 2020).

The Role of Peatlands in Ecosystem Conservation

A total of 44 respondents agreed that peatlands played a significant role in regulating environmental balance, particularly in their function as a carbon store and in maintaining water table balance (Kwon et al., 2022). This opinion is in line with the ecological role,

as the largest reservoir of water and carbon on Earth for thousands of years. However, unsustainable peatland management practices, such as drainage and burning, release substantial amounts of stored carbon into the atmosphere, thereby contributing significantly to greenhouse gas emissions (Ruwaimana et al., 2020). The respondents viewed the introduction of paludiculture as a pragmatic management solution, because the model maintains the ecological functions of peatlands while generating direct economic benefits for local communities through the sustainable production of high-value commodities.

Although agroforestry on peatlands holds great potential, there are several important challenges for its implementation. First, farmers were not aware of other combinations of commodities. A large proportion of respondents admitted to not being aware of alternative commodity integration models for peatland cultivation, reflecting a significant gap between information and technical capacity. This limitation is a major obstacle to the expansion of paludiculture systems. Farmers are generally not enthusiastic to adopt new practices without a clear understanding of their management requirements and expected benefits (Ziegler et al., 2021).

To address this challenge, continuous extension services and capacity-building programmes are needed to strengthen farmers' knowledge and practical skills in adopting sustainable agricultural practices appropriate for peatland ecosystems (Choi et al., 2024). Second, another challenge is water management in peatland areas. A total of 49 respondents agreed that improvement of water management has the potential to enhance community welfare. Among the characteristics of peatlands is the natural ability to retain water, which offers opportunities to support aquaculture and agricultural irrigation systems (Hairani & Noor, 2020). Good management has been shown in previous studies to be important in reducing the risk of fires and facilitating the successful implementation of paludiculture systems (Tan et al., 2021). These systems require high stability of the groundwater table to maintain ecological balance and support sustainable land productivity. Therefore, strengthening capacity in water management is a strategic aspect of developing sustainable agriculture based on the ecosystems (Thornton et al., 2020). Third, Land Burning Risk, a total of 38 respondents acknowledged that peatland burning practices are recognised as the primary cause of environmental degradation. Despite being a common method for land clearing among some farmers, it reduces soil fertility, damages the peatland ecosystem structure, and releases large quantities of greenhouse gas emissions into the atmosphere (Sofiyuddin et al., 2021). Most peatlands that have been drained and converted for agricultural use still hold potential for transition into paludiculture systems. Approximately one-third of the total swamp area can be optimised for various types of paludiculture. Furthermore, the biodiversity characteristic of swamp ecosystems tends to increase when rewetting (restoring wet conditions) and the practices are implemented, compared to conditions where the land remains dry or degraded (Munawar et al., 2024).

Paludiculture, which avoids burning, offers a more environmentally friendly solution to enhance productivity without damaging ecosystem structure (Budiman et al., 2020). Fourth, Peatlands as Marginal Land, a total of 41 respondents stated that peatlands are classified as marginal lands with low fertility levels. The soils are typically highly acidic and deficient in essential nutrients, thereby requiring adaptive cultivation methods. The paludiculture model offers a viable solution to these limitations through the use of diverse commodities that are capable of thriving in marginal conditions while also providing economic benefits (Wicaksono & Zainal, 2022).

The overall results show that the communities in the South Sumatra areas support the use of peatlands through the paludiculture method to enhance agricultural productivity while maintaining the sustainability of the ecosystem. However, several important challenges were observed, including the limited knowledge of paludiculture practices among farmers and the continuing threat of land burning. These constraints can be overcome by sustained educational efforts, as well as promotion of more environmentally sustainable land management practices. The adoption of paludiculture supported by government and academic institutions offered a promising solution to improve economic well-being and ensure the long-term conservation of peatland ecosystems.

Analysis of Farmers' WTI in Agroforestry Models to Optimise the Sustainability of Agricultural Businesses in Peatlands

Table 4 explains the distribution of farmers' WTI in the agroforestry model as a method for sustainable peatland management. The majority of respondents (72.09%) showed WTI, while 27.91% were not willing. This distribution reflected a reasonably good level of acceptance of the suggested agroforestry model by farmers. Investment decisions were largely influenced by perceptions of the commodity diversification opportunities offered by agroforestry systems, which allowed for more productive land use compared to conventional monoculture systems (Belay & Melka, 2024). Farmers tended to favour annual monoculture systems due to the quicker financial returns and lower short-term risks. However, these benefits were projected to decline in the long term as a result of unsustainable land use practices (Goswami et al., 2024).

The innovative commodity combination model within agroforestry was considered to have strategic potential for expanding farmers' economic base, particularly in peatland areas that possess unique ecological characteristics and high vulnerability to degradation. By integrating multiple commodities, including food crops, forestry species, and aquaculture, this model enhances farmers' income diversification, strengthens food security, and promotes more environmentally sustainable agricultural practices. Its flexibility in adapting to the biophysical conditions of peatlands makes the model highly relevant for implementation in landscape-based agriculture that is resilient to climate change and environmental crises (Nugroho et al., 2025).

Table 4
Farmers' WTI

WTI	Frequency (%)	Reasons	Frequency	Percentage (%)
Yes	62 (72.09%)	Product Diversifications	27	42.86
		Sharing Production Costs for Total Cost Decreasing	9	14.29
		Higher Income	9	14.29
		Decreasing Land Burning	18	28.57
		Total	62	100.00
No	24 (27.91)	Limited Financial Resources	15	63.79
		Absence of Governmental Assistance	1	5.17
		Ignorance of Knowledge and Experience	5	20.69
		Decreasing of Agriculture Land and Labour	2	10.34
		Total	24	100.00

The study showed significant structural barriers to the model implementation. Most farmers who were unwilling to invest cited limited access to financial resources as the primary constraint (Goswami et al., 2024). A lack of capital not only obstructs the initial development of agroforestry systems but also limits farmers' capacity to access technology, training, and other productive inputs (Castle et al., 2021). Therefore, policy interventions that facilitate access to financing, provide investment subsidies or incentive schemes, and strengthen institutional capacity are crucial prerequisites for promoting the expansion and long-term sustainability of this model at the grassroots level.

Table 4 shows that the majority of farmers at 72.09 % were WTI. This interest was driven primarily by commodity diversification, increased income, and production cost efficiency. Agroforestry is also perceived as being able to reduce land burning practices (28.57%), which is relevant in the context of sustainable peatland management. Conversely, approximately 27.91% of farmers were unwilling to invest. This is primarily due to limited financial resources (63.79%), followed by lack of knowledge and experience, limited labour and land, and minimal government support. These results confirmed that investment decisions were more influenced by economic factors and knowledge capacity than other structural factors. Therefore, policy interventions need to focus on improving access to financing, extension services, and strengthening farmer institutions.

In the binary logistic regression model in Table 5, the Pseudo R^2 value for the 13 explanatory variables was 0.31, reflecting a moderate level of explanatory power. The log pseudo-likelihood value of 40.66 showed that the WTI was significantly different from zero, and the applied model provided a better fit compared to the null model. Therefore, the estimation of farmers' WTI is considered valid as a basis for further analysis (Wiradanti et al., 2020).

Table 5
Binary logistic regression for farmers' WTI in implementing various agroforestry models on peatland

Variables	Coef.	Wald	Exp(B)	Sig
(X1) Age	0.0344	0.050498	1.0350	0.496
(X2) Indigenous people	3.2367	1.793711	25.4498	0.071*
(X3) Gender	-0.7021	1.273711	0.4956	0.581
(X4) Education	1.3616	0.76561	3.9026	0.075*
(X4) Family size	-0.4353	0.320494	0.6471	0.174
(X5) Land productivity	0.6793	1.952534	1.9725	0.728
(X6) Land scale	0.5834	0.420857	1.7921	0.166
(X7) Land certificate	0.0880	0.887793	1.0920	0.921
(X8) Dominant land for agriculture	-4.1969	1.624618	0.0150	0.010***
(X9) Dominant land for plantation	-3.1388	1.77135	0.0433	0.076*
(X10) Dominant land for forestry	-1.1994	1.300358	0.3014	0.356
(X11) Having a plan for increasing productivity	1.3193	2.777069	3.7408	0.001***
(X12) Knowledge about peatland management	-1.4580	2.410044	0.2327	0.001***
(X13) Member of farmer organisation	0.7374	1.136898	2.0904	0.017**
Constanta	1.9883	6.846975	7.3032	0.772

Note. Range interval. * p < 0,1; ** p < 0,05; *** p < 0,01

Among the variables representing demographic characteristics, indigenous status ($p < 0.1$) was discovered to significantly influence farmers' WTI in implementing various agroforestry commodity combination models. In this case, 96% of the respondents identified as indigenous people and expressed willingness to allocate funds toward innovative agroforestry systems. Farming experience in the region also influenced WTI, and indigenous community members holding traditional authority and responsibilities show greater willingness than migrants. Indigenous communities play a crucial role in environmental and territorial governance because of close interactions with local ecosystems and community networks. These communities frequently serve as trusted sources of information, influence public land-use decisions, and promote the adoption of sustainable land-use practices. Indigenous knowledge and leadership further support the development of climate-resilient, environmentally sustainable food production strategies. Another demographic characteristic with a significant influence was the level of education. The higher the educational attainment, the greater the possibility of adopting agroforestry models in peatlands ($p < 0.1$). Individuals with longer years of schooling are generally more open to innovation and more receptive to agroforestry practices (Buyinza et al., 2020)**.** However, other variables such as age, gender, household size, land productivity, land area, and land tenure status did not show significant influence within the model. This is because the variables do not directly increase adaptive capacity or reduce business risks in peat ecosystems with high biophysical uncertainty and are relatively uniform across

farmers. The factors do not influence investment decisions strongly enough compared to more decisive aspects such as knowledge and institutional support.

From the perspective of farming characteristics, farmers engaged in intensive land use for food crop production ($p < 0.01$) and timber plantation purposes ($p < 0.1$) were generally less WTI in innovative agroforestry systems. The individuals may be satisfied with the current systems and not want to introduce new practices that may disrupt the established way of working. This is consistent with previous studies where the adoption of new agricultural practices by farming households with high land productivity is less possible. Farmers who wanted to increase productivity in their region ($p < 0.01$) also had less tendency to adopt commodity combination models. This showed that the local community does not view the models as having the potential to enhance productivity. These results are consistent with the perception that innovative agroforestry in peatlands is not considered a productive or viable alternative. Expectations about the future of the farm and the probability of succession over generations also appear to influence investment decisions.

The expectation may be mediated by the length of residence in a given area. Families that are multi-generational and have lived in the area for a long time tend to be more attached to the land and have longer-term aspirations. Therefore, other factors such as the age of the household head, number of descendants, and the possibility of intergenerational continuity may be important in sustaining farming activities over time.

Technical knowledge about various agroforestry models had a negative effect on WTI ($p < 0.01$). This result suggests that farmers with better knowledge of peatland management tend to evaluate agroforestry more critically and cautiously. Those with dominant land use for agriculture (Coef. = -4.1969; $p < 0.01$) and plantations (Coef. = -3.1388; $p < 0.1$) also showed lower WTI, possibly due to greater awareness of the capital requirements, management complexity, and long-term sustainability challenges related to agroforestry implementation. Therefore, higher knowledge does not always increase WTI, but may reflect more rational and risk-aware decision-making.

A negative relationship was observed between agroforestry knowledge and WTI. This suggests that higher knowledge does not necessarily translate into higher investment intention. Instead, farmers with better understanding tend to evaluate agroforestry more critically, particularly in terms of capital requirements, management complexity, and long-term risk-return trade-offs. This result should not be interpreted as a perception that agroforestry is unsustainable, but reflects more cautious and realistic investment behaviour under existing constraints such as limited capital access, opportunity costs, and reliance on established land-use systems. Accordingly, the study emphasises heterogeneity in investment decision-making and bounded rationality among farmers in assessing new land-use innovations.

Several successful community-based agroforestry practices in Indonesia support the feasibility of the proposed model in this study. For example, agroforestry systems in the *Gunung Salak* Valley, West Java, have successfully combined fruit trees, timber trees, and agricultural crops, contributing to household income while maintaining biodiversity and ecosystem sustainability. These results showed that community-based agroforestry can serve as a realistic and sustainable land management strategy (Rahman et al., 2021).

Costs and Benefits Insight of Several Agroforestry Models

Table 6 provides a clear and structured comparative overview of the differences in characteristics, costs, and benefits of each agroforestry model. By presenting aspects of components, economic life, inputs, and production outputs, the table helps explain the economic and technical basis underlying farmers' investment choices. Furthermore, it strengthens the analysis by showing how the level of diversification affects potential profits and risks, providing empirical evidence to support the discussion of factors influencing WTI. The existence of this table is important to increase the clarity, validity, and depth of the analysis in explaining the relative advantages of each agroforestry model on peatlands. Table 6 is the detailed comparison of the agroforestry models.

Table 6
Comparative overview of agroforestry models: Agrosilviculture, agrosilvofishery and agrosilvopastural

Aspect	Agrosilviculture	Agrosilvofishery	Agrosilvopastural
Main Components	Integration of forestry and seasonal agricultural crop cultivation.	Integration of forestry, agricultural system, and aquaculture activities.	Integration of forestry, agricultural systems, and livestock husbandry.
Commodities	*Meranti*, Pineapple, Rice Farming	*Jelutung*, Pineapple, Catfish	*Nyamplung*, Rice Farming, Duck eggs, Duck
Land Area	1 Hectare	1 Hectare	1 Hectare
Economic Life	10 Years	30 Years	35 Years
Discount Rate	7%	7%	7%
Planting Quantity	342 *Meranti* trees 7,874 Pineapple Seedlings 40 Kg/ha Rice seeds	368 *Jelutung* trees 8,647 Pineapple seedlings 288,000 Catfish Fingerlings	400 *Nyamplung* trees, 40 Kg/ha rice seeds, 7,412 ducks, 100 ducklings
Final Production Output	311m^3 *Meranti* Trees 7,412 Pineapples 1,600 Kg/ha Rice	236m^3 *Jelutung* Trees 8,235 Pineapples 36,000 Kg/ha Catfish	42 Tons Nyamplung Fruits 1,600 Kg/ha rice 13,650 Duck eggs 170 Duck
Planting Spacing	*Meranti*: 3×8m Pineapple: 1×1m Rice: 25×25cm	*Jelutung*: 3×8m Pineapple: 1×1m Pond: 9 Units (9×4m)	*Nyamplung*: 5×5m Rice: 25×25cm Duck Pen: 10 Units (10×2m)

All three models integrate forestry components with agricultural activities but have varying levels of diversification. The agrosilviculture model combines forestry crops such as *meranti* with annual crops (pineapples and rice), leading to a moderate level of diversification with a relatively short economic life (10 years). Meanwhile, agrosilvofishery integrates a fisheries component, contributing to a more complex system with a longer economic life (30 years) and a wider variety of outputs, including timber, food crops, and fish. The agrosilvopastural model is the most diversified system, integrating forestry, food crops, and livestock. It has the longest economic life (35 years) and produces a variety of products such as timber, rice, meat, and eggs.

Economically, all three models apply the same discount rate but differ in planting density and production volume, reflecting varying levels of land-use intensity and economic returns. Agrosilvofishery and agrosilvopastural models have higher output volume and diversity (Chappa et al., 2024), potentially increasing income stability and reducing business risks compared to agrosilviculture. Both models also include higher levels of management complexity and input requirements. The higher the level of diversification of agroforestry systems, the greater the potential economic and ecological benefits (Raj et al., 2022), but they require greater technical capacity and investment readiness from farmers.

Financial Feasibility of Agroforestry Model

The analysis of the financial profitability of an agroforestry business aims to determine the potential profit, which is subsequently associated with performance. To assess this profitability, the study relies on various investment criteria, including the Net Present Value (NPV) method, the Internal Rate of Return (IRR), the benefit-cost ratio (B/C ratio), and the payback period. For an operation to be considered profitable, NPV, IRR, and B/C ratio shall exceed zero, the interest rate, and one (B/C > 1), respectively. Additionally, the payback period should be shorter than the investment's useful life. The corresponding discount factor is used to calculate the NPV, as detailed in Table 7.

NPV represents the difference between benefits and costs and serves as an investment criterion to measure the feasibility of a business. Table 7 shows that agrosilviculture (*meranti*-pineapple-rice), agrosilvopastural (*nyamplung*-rice-duck), and agrosilvofishery (*jelutung*-pineapple-catfish) are economically viable. This is confirmed by the NPV calculation, which produced a positive value based on an interest rate of 7%. In concrete terms, this implied that an investment in a meranti-pineapple-rice agrosilviculture business generates a profit of IDR266,137,167. Meanwhile, for *nyamplung*-rice-duck agrosilvopastural and agrosilvofishery businesses, the profits amount to IDR1,010,975,032 and IDR1,637,114,339, respectively.

Table 7
Financial feasibility analysis of agroforestry model

No.	Agroforestry Model	Component	Economic Life Scale (Years)	Discount Factor (%)	NPV (IDR)	IRR (%)	B/C Ratio	Payback Period (Years)
1	Agrosilviculture	*Meranti* - Pineapple - Rice	10	7	266,137,167	20	2,01	4.92
2	Agrosilvofishery	*Jelutung* - Pineapple - Catfish	30	7	1,637,114,339	33	1,09	5.50
3	Agrosilvopastural	*Nyamplung* - Rice - Duck	35	7	1,010,975,032	29	2,60	3.28

The financial indicators in the form of IRR for each type of agroforestry were 20%, 33%, and 29%, exceeding the discount rate of 7%. This implied that agrosilviculture, agrosilvofishery, and agrosilvopastural businesses were categorised as feasible to be developed. IRR shows the ability to earn income from invested capital. Agrosilvofishery with a combination of *jelutung* - pineapple - catfish components had the highest NPV and IRRl values compared to other combinations. In terms of the B/C ratio calculation with an interest rate of 7%, the value for each type of agroforestry was more than one (B/C ratio> 1). This implied that agrosilviculture, agrosilvofishery, and agrosilvopastural businesses were profitable and feasible to run. Therefore, the three types of agroforestry are expected to remain economically viable because the B/C ratio value obtained was quite significant.

Based on the payback period calculation, representing the time required to recoup investment expenditures, it takes agrosilviculture, agrosilvofishery, and agrosilvopastural 4.92 years (4 years 335 days), 5.50 years, and 3.28 years (3 years 102 days), respectively, to recover the investment costs. Given that these payback periods are shorter than their respective maximum economic lifespans (10, 35, and 30 years), the investment projects for agrosilviculture, agrosilvofishery, and agrosilvopastural are feasible and acceptable.

In general, the three types of agroforestry modalities proved to be profitable and advantageous. This is because agroforestry integrates diverse agricultural components, including crops, livestock, and fisheries, thereby increasing overall productivity and improving harvest yields. The high yields achieved through these systems contribute to increasing farmers' income. The agroforestry system offers great potential for environmental conservation and promotes human welfare (Hasannudin et al., 2022). From an economic perspective, this system offers farmers greater income-generating opportunities by enabling the cultivation of high-value crops and the integration of profitable livestock or aquaculture enterprises.

Based on field observations, farmers in the study area generally rely on diversified livelihood strategies to maintain household income during the waiting period before harvest. In addition to farming activities such as rubber and oil palm cultivation, several respondents also worked as labourers, traders, construction workers, motorcycle taxi drivers, teachers, and civil servants. This shows that livelihood diversification plays an important role in supporting household economic resilience while waiting for agroforestry commodities to generate income. Limited financial capacity during the early implementation stage remains a major challenge for smallholder farmers adopting agroforestry systems.

The results of this study show that various sociodemographic factors significantly influence smallholder farmers' willingness to adopt agroforestry practices. Family members, particularly the household head and spouse, play a significant role in shaping investment

intentions toward the agroforestry model. Certain aspects related to wood production shape how education level and experience affect the adoption of these practices. The study shows that indigenous populations also invest in the agroforestry model, possibly due to deep knowledge of the region and its resources. Specific agricultural characteristics have a negative impact on the adoption of agroforestry practices (Quaye et al., 2021). Farmers who manage their land by prioritising productivity and combining agricultural crops with tree plantations showed a greater tendency not to adopt agroforestry systems (Jordon et al., 2023). This result is in line with previous reports stating that economic incentives and resource-related factors can negatively influence the adoption of agroforestry systems (Autio et al., 2021; Bist et al., 2024). Furthermore, knowledge of technical aspects negatively impacts farmers' WTI (Li et al., 2021; Ranacher et al., 2021). This can be attributed to the perception of difficulties or impracticalities regarding the techniques. The results emphasise the importance of developing effective teaching methods. Building confidence in the benefits of the agroforestry system is also crucial for the adoption of these practices. Finally, the perception of improved well-being resulting from innovative agroforestry practices positively influences the farmers' willingness. This underscores the importance of communicating the system's potential health and environmental benefits (Ntawuruhunga et al., 2023).

In light of these results, the following proposals can be formulated to foster the adoption of agroforestry systems in support of low-carbon agriculture and improved livelihoods: (1) Target decision-makers: to increase the tendency of adoption, extension services should prioritize collaboration with heads of households and their spouses, who hold decision-making power within the family (Antwi-Agyei & Stringer, 2021; Lecoutere et al., 2023); (2) Foster social proximity: encourage the participation of indigenous groups and local resource users, given that these actors are more inclined to invest in agroforestry practices (Cancino et al., 2022); (3) Simplify technical information: develop effective communication strategies to dispel misconceptions regarding the complexity of agroforestry systems and facilitate understanding of their functioning and benefits (Kumar, 2025); (4) Prioritize well-being: prove how these systems can improve community health and well-being—aspects the community values more than economic gains (Rahman et al., 2021); and (5) Address productivity: provide evidence of the effectiveness of agroforestry methods in boosting agricultural productivity (Noeldeke et al., 2022).

This study has certain limitations that need to be addressed. The 86 farmers in the sample may not accurately reflect the entire population of smallholders in the region. Furthermore, the cross-sectional nature of the data limits the ability to establish causal relationships between the factors analysed and the willingness to adopt the practice. Future studies should rely on longitudinal data to better understand the dynamics of this adoption process over time and use larger, more diverse participant samples to validate

the results. For policymakers and practitioners seeking to promote agroforestry models in Indonesia, analysing the impact of incentive programs or specific policy measures on adoption rates could also provide valuable insights. Further study is needed to identify peatland agroforestry models that are widely accepted by farmers, along with the barriers to their development and compatible local cultural practices, to determine the most suitable method for implementation.

The results of this study have significant implications for the development and implementation of the innovative commodity combination restoration model as a means to promote low-carbon agriculture and improve livelihoods in Indonesia. By targeting decision-makers, promoting social proximity, simplifying technical information, emphasising welfare benefits, and addressing productivity issues, extension services as well as policymakers can better support the implementation of the agroforestry model practices among smallholder farmers. This will then contribute to ongoing efforts to restore degraded peatlands, mitigate climate change, and improve the well-being of local communities.

CONCLUSION

In conclusion, the majority of respondents agreed that productivity can be increased through effective utilisation of peatlands by combining many alternative commodities in agroforestry schemes. The combination of agrosilviculture, agrosilvofishery, and agrosilvopastural on wetlands led to increased farmer incomes while preserving environmental sustainability. The study showed that the respondents also agree that the integration of native trees or other suitable plants with agricultural products increased economic value and lowered the danger of peatland degradation.

Approximately 72.09% of WTI respondents expressed a willingness to implement the proposed agroforestry model. The results identified several significant determinants of WTI. Indigenous status, education level, plans to increase land productivity, and participation in farmer groups showed positive associations. Meanwhile, dominant land use for agriculture and plantations, as well as agroforestry-related knowledge, showed significant negative associations. Other variables, including age, gender, household size, land productivity, and land ownership, were not significantly associated with WTI. Economic analysis confirmed that the agrosilvofishery and agrosilvopastural models require greater investment than the agrosilviculture model. Among the three scenarios, agrosilviculture generated the highest profitability.

The results provided valuable insights for developing and implementing innovative commodity-based restoration models that support low-carbon agriculture while improving rural livelihoods in Indonesia. Governments and extension services promoted agroforestry adoption among smallholder farmers by targeting key decision-makers, strengthening farmer organisations, simplifying technical information, emphasising livelihood benefits,

and addressing productivity constraints. These efforts contributed to climate change mitigation, peatland restoration, and improved community welfare.

This study had several limitations, particularly related to its geographically specific scope and the potential constraints of the sample size, which may affect the generalizability of the results. Therefore, future study is recommended to expand the geographical coverage, increase the sample size, and test the model across different ecosystem conditions as well as commodity types in order to enhance the external validity of the results.

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Wild Gingers of Malaysia (Zingiberaceae Martinov): A Source of Bioactive Phytocompounds with Promising Antioxidant and Antidiabetic Potentials

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ABSTRACT

Oxidative stress contributes to chronic diseases including diabetes, cardiovascular disorders and neurodegeneration. Although Zingiberaceae species are valued in ethnomedicine, wild Malaysian gingers remain underexplored for their therapeutic properties, particularly from leaves and stems. This study investigated the phytochemical composition, antioxidant and α-glucosidase inhibitory activities of leaves, stems and rhizomes from seven wild Zingiberaceae species that were collected from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. Total phenolic content (TPC), total flavonoid content (TFC),

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and total terpenoid content (TTC) were quantified using colourimetric assays. Antioxidant activities were assessed via DPPH, ABTS, FRAP and nitric oxide (NO) scavenging assays. The α-glucosidase inhibition was evaluated using enzyme-based colourimetric assays. Leaves consistently exhibited the highest TPC (up to 57.88 mg GAE/g DW), TFC (72.83 mg RE/g DW) and TTC (0.08 mg LE/g DW), followed by rhizomes and stems. Antioxidant assays showed leaves had superior radical scavenging in ABTS and FRAP assays, rhizomes showed stronger DPPH activity, while stems showed the least activity. Correlation analysis indicated that phenolic compounds were the primary contributors to antioxidant activity. α-Glucosidase inhibition was only detected in rhizomes of *Boesenbergia plicata* (IC_{50} = 16.77 µg/mL) and *Sundamomum hastilabium* (IC_{50} = 20.26 µg/mL), comparable to cultivated Zingiberaceae species. Wild Malaysian ginger leaves were rich sources of antioxidant phytochemicals, while rhizomes of selected species showed promising antidiabetic activity. These findings highlight the therapeutic potential of underexplored Zingiberaceae species and support sustainable utilisation beyond rhizomes.

Keywords: α-glucosidase inhibition, antioxidant activity, phytochemical profiling, wild ginger Malaysia, wild Zingiberaceae

INTRODUCTION

Oxidative stress is a major factor in the development of chronic diseases such as diabetes mellitus, cardiovascular disorders, and neurodegenerative conditions (Reddy, 2023). Excessive generation of Reactive Oxygen Species (ROS) can disrupt cellular redox homeostasis, resulting in oxidative damage to proteins, lipids, and nucleic acids, which ultimately contributes to inflammation and metabolic dysfunction (Liguori et al., 2018). Therefore, natural antioxidants derived from plants have gained considerable attention as safer and more biocompatible alternatives to synthetic agents for preventing and managing oxidative stress-related diseases. Phenolic compounds and flavonoids present in Zingiberaceae species have attracted considerable interest due to their potent antioxidant and antidiabetic properties. For example, *Kaempferia galanga* has demonstrated strong DPPH and ABTS radical scavenging activities that are related to its rich phytochemical composition (Nonglang et al., 2022). Similarly, essential oils from *K. galanga* exhibited both in vitro and in vivo antioxidant activities (Wang et al., 2023b). In addition, bioactive constituents isolated from *Alpinia galanga* have shown alpha-glucosidase and protein tyrosine phosphatase 1B (PTP1B) inhibitory activities, which are potentially related to diabetes management (Liu et al., 2023). These previous findings highlight Zingiberaceae species as promising natural alternatives for mitigating oxidative stress and metabolic disorders.

With approximately 1,449 accepted species across 56 genera, the Zingiberaceae family is highly diverse in Southeast Asia and is particularly valued in ethnomedicine for its antioxidant, anti-inflammatory, and antidiabetic properties (Sedek et al., 2024).

Cultivated species such as *Zingiber officinale* (ginger), *Curcuma longa* (turmeric) and *Kaempferia galanga* (kencur) specifically have been extensively studied and demonstrate significant antioxidant activity and glucose-lowering effects due to their high content of phenolic, flavonoid, and terpenoid constituents. For instance, gingerol and shogaol from *Z. officinale* and curcuminoids from *C. longa* are well-known active compounds with antioxidant and antidiabetic activities (Gu et al., 2024; Mashhadi et al., 2013). However, the majority of these studies focus on the rhizome and frequently ignore other plant parts that may have medicinal significance. Recent studies have shown that aerial parts of cultivated Zingiberaceae species are valuable sources of phytochemicals. For instance, leaves of *C. longa* were reported to contain abundant terpenoids and exhibit considerable antioxidant activities (Braga et al., 2023). Similarly, postharvest residues and shoots of *Z. officinale* retained substantial antioxidant capacity (Jorge-Montalvo et al., 2023). Other than that, strong antioxidant properties were reported from essential oils of *K. galanga* in both in vitro and in vivo studies, indicating that bioactive constituents are not limited to rhizomes alone (Wang et al., 2023b). This suggests that relying solely on rhizomes might ignore other plant parts with equal or greater therapeutic potential, as well as contribute to unsustainable harvesting practices.

Despite the extensive utilisation of cultivated gingers, the phytochemical diversity and biological activities of wild Malaysian Zingiberaceae remain poorly explored. Species such as *Boesenbergia plicata* (Ridl.) Holttum, *Boesenbergia prainiana* (King ex Baker) Schltr., *Globba patens* Miq., *Hornstedia leonurus* (J. Koenig) Retz., *Scaphochlamys klossii* (Ridl.) Holttum, *Sundamomum hastilabium* (Ridl.) A.D. Poulsen & M.F. Newman, and *Amomum* sp., have received limited scientific attention. Recent metabolomic studies have shown that wild relatives of cultivated gingers may possess unique chemical profiles shaped by ecological adaptation and environmental factors. According to Singh et al. (2025), there were substantial differences in the composition of secondary metabolites between wild and cultivated Zingiberaceae, including phenolic derivatives, terpenoids, and other specialised metabolites. This study strongly suggests that wild relatives of cultivated gingers are chemically unique resources with untapped medicinal potential. Similar studies conducted in other Southeast Asian countries such as Thailand and Indonesia have demonstrated considerable variations in phytochemical composition and antioxidant activity not just among different species but also among plant organs. This suggests that leaves and stems may contain bioactive compounds similar to those found in rhizomes (Hariyanto et al., 2024; Uthumporn et al., 2016). Nonetheless, comprehensive comparative investigations involving various plant organs across wild Malaysian Zingiberaceae species remain scarce. Therefore, a systematic evaluation of phytochemical contents and biological activities among different plant parts is necessary to identify promising species and support the sustainable utilisation of Malaysia's native ginger resources.

Therefore, this study aims to address this knowledge gap by evaluating the phytochemical content and bioactivities within leaves, stems and rhizomes of seven wild Zingiberaceae species from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. In each sample, the total phenolic content (TPC), total flavonoid content (TFC), and total terpenoid content (TTC) are measured. These profiles are then correlated with robust antioxidant (DPPH, ABTS, FRAP, and NO) and antidiabetic assays. Ultimately, this study aims to identify promising species and plant parts that may serve as a new source of natural antioxidants and antidiabetic agents while also contributing to sustainable resource utilisation in Malaysian forests.

MATERIALS AND METHODS

Sample Collection and Preparation

The study was conducted in Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia, which are characterised as humid lowland rainforest with shaded areas and rocky stream areas near waterfalls. Wild ginger species were sampled from suitable habitats including stream banks, swampy areas, and lowland forests at 200-500 m above sea level (a.s.l.) (Sedek et al., 2024). Six wild ginger species were collected from Panti Forest Reserve (Kota Tinggi, Johor, Malaysia) and one species from Endau-Rompin National Park (Johor, Malaysia). All specimens were taxonomically identified and deposited into the herbarium in Universiti Tun Hussien Onn Malaysia (UTHM) with assigned voucher numbers (*S. klossii* - SM0079; *G. patens* - SM0088*; B. prainiana* - SM0090*; S. hastilabium* - SM0091*; B. plicata* - SM0102*; Amomum* sp. - SM0103; and *H. leonurus* - SM0104) (Figure 1, Figure 2). Plant collection was conducted with permission from the relevant authorities and in accordance with applicable local regulations governing access to forest reserves and protected areas in Johor, Malaysia. For extraction preparation and phytochemical analysis, healthy and fully expanded mature leaves, together with mature stems and rhizomes, were collected from each species. To minimise variation associated with plant developmental stage, all plant materials were sampled from mature individuals at the flowering stage or post-vegetative stage. The rhizomes, stems and leaves of the seven wild ginger species were cleaned and thoroughly rinsed under running tap water to remove any debris and soil. Next, the cleaned samples were sliced into small pieces and dried at 60 °C using the oven-drying method for 72 hours (Uthumporn et al., 2016). After drying, the samples were ground into powder form, sealed properly, and stored in a freezer at -20 °C for further use.



Figure 1. Wild ginger species (Zingiberaceae) collected from Southern Malaysian Forest Reserva: Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia: (A) *Boesenbergia plicata*; (B) *Boesenbergia prainiana*; (C) *Globba patens*; (D) *Scaphochlamys klossii*; (E) *Sundamomum hastilabium*; (F) *Hornstedtia leonurus*. The figure highlights the inflorescence, which was crucial for species identification



Figure 2. Overall vegetative morphology of selected wild ginger species (Zingiberaceae) collected from Southern Malaysian Forest Reserves (Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia). Distinct variations in leaf lamina architecture, surface texture, and vegetative habits are observed among the species: (A) *Boesenbergia plicata* (Ridl.) Holttum, demonstrating characteristic deeply plicate, corrugated leaf blades; (B) *Boesenbergia prainiana* (King ex Baker) Schltr., showing a typically monophyllous, broad-ovate leaf habit; (C) *Globba patens* Miq., exhibiting slender, distichous pseudostems with thin, lanceolate leaf blades and undulate margins; (D) *Scaphochlamys klossii* (Ridl.) Holttum, displaying a low-growing terrestrial habit with prominent parallel-pinnate venation; (E) *Sundamomum hastilabium* (Ridl.) A.D. Poulsen & M.F. Newman, indicating an oblong-lanceolate lamina with a long-acuminate apex; (F) *Amomum* sp., illustrating typical large, frond-like forest-canopy pseudostems: and (G) *Hornstedtia leonurus* (J.Koenig) Retz., showcasing robust, coriaceous leaves with elongated, rigid lligules
Source: Taxonomic descriptions and authorities adapted in part from Holttum, 1950; Larsen & Mood, 1998; Sedek et al., 2024, Turner, 1995)

Ethanolic Extraction of Plant Sample

The ethanolic extraction process for each plant part (rhizomes, stems, and leaves), began by weighing 2 g of each dried powder sample and placing them into separate conical flasks. The samples were extracted using 40 mL of absolute ethanol and left for 5 hours at room temperature while being stirred with a magnetic stirrer. Then, the extract was filtered using Whatman filter paper and concentrated using a solvent concentrator for 3 hours at 45°C to obtain a crude extract. The crude extract was then redissolved in absolute ethanol to 8000ppm (8 mg/mL) concentration and stored at 4°C for further use.

Phytocompound Characterisation

Determination of Total Phenolic Content (TPC)

The TPC in leaves, stems, and rhizomes of the wild ginger species was measured separately using the Folin-Ciocalteu method, as described by Sharifi-Rad et al. (2017). The ethanolic extract of each sample (20 µL) was pipette-transferred into a 96-well microplate, and 100 µL of the 10% Folin-Ciocalteu (v/v) reagent was added, and the mixture was incubated for 5 minutes. After 5 minutes, 80 µL of the 7.5% (w/v) sodium carbonate solution (Na_2CO_3) was added, and the mixture was further incubated for 30 minutes. Finally, the absorbance of the mixture was measured at 760 nm using a microplate reader (VersaMax ELISA, United States). The TPC was reported as milligrams of gallic acid equivalents per gram of dry weight sample (mg GAE/g DW). Gallic acid was obtained from Sigma-Aldrich (Hong Kong, China).

Determination of Total Flavonoid Content (TFC)

The TFC in leaves, stem and rhizomes of the wild ginger was measured separately by using the aluminium chloride calorimetric method as described by (Ali et al., 2018) with some modifications. The ethanolic extract of each sample (50 µL) was pipetted and transferred into a 96-well microplate, and 100 µL of 95% ethanol and 20 µL of 10% aluminium chloride [$Al(NO_3)_3$] were added, and the mixture was incubated for 3 minutes in the dark at room temperature. After incubation, 20 µL of the 1.0 M sodium acetate ($C_2H_3NaO_2$) solution was added, and the mixture was further incubated for 40 minutes. Finally, the absorbance of the mixture was measured at 415 nm using a microplate reader (VersaMax ELISA, United States). The TFC was reported as milligrams of rutin equivalents per gram of dry weight sample (mg RE/g DW). Rutin was obtained from Sigma-Aldrich (Hong Kong, China).

Determination of Total Terpenoid content (TTC)

The TTC in leaves, stems, and rhizomes of the wild ginger was measured separately by following the protocol established by Biswas et al. (2021) with slight modifications. The ethanolic extract (200 µL) of each extract sample was mixed with 1 mL of perchloric acid ($HClO_4$) and 300 µL of 5% vanillin solution, followed by 5 mL of glacial acetic acid (CH_3COOH) to the mixture. The mixture was incubated in the dark at room temperature for 45 minutes, and the absorbance of the extract was measured at 548 nm using UV-Vis spectrophotometer (Agilent, United States). TTC was expressed as milligrams of linalool equivalent per gram of dry weight sample (mg LE/g DW). Linalool was obtained from Sigma-Aldrich (Hong Kong, China).

Antioxidant Activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

DPPH radical scavenging assay was determined to assess the antioxidant capacity in accordance with the method described by Idris et al. (2019) with some modification. The ethanolic extract of each sample 200 µL was pipetted and transferred into a 96-well microplate, and 100 µL of ethanol was mixed with the sample using the serial dilution method. Then, 100 µL of ethanolic solution of 0.1 mM DPPH radical solution was added and incubated for 30 minutes in the dark at room temperature. The absorbance of the mixture was measured at 515 nm using a microplate reader (VersaMax ELISA, United States). Inhibition (%) of the extracted sample was calculated using the following Equation 1:

$$DPPH\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \qquad [1]$$

where $A_{control}$ is the absorbance of the DPPH solution without antioxidant and A_{sample} is the absorbance of the sample containing antioxidant. The antioxidant activity of each extract sample was expressed as the half maximal inhibitory concentration (IC_{50}) value (mg/mL).

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

This assay was conducted following the protocol outlined by Jimoh et al. (2019) with some modifications. The ABTS radical was prepared by mixing equal volumes of 2.45 mM potassium persulfate ($K_2S_2O_8$) and 7 mM ABTS solution prepared in ethanol. The mixture was kept in the dark for 12-18 hours at room temperature and then diluted with ethanol at a 1:50 ratio until an absorbance of 0.700±0.003 was achieved at 734 nm. Then, equal volumes of plant extracts and standard drugs at various concentrations were

mixed with ABTS (1:1 v/v), left in the dark for 6 minutes, and the absorbance was measured at 734 nm. The percentage of ABTS scavenging activity of the crude extracts and standards (rutin) was calculated using the following Equation 2:

$$ABTS\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \quad [2]$$

where $A_{control}$ is the absorbance of the ABTS solution without antioxidant and A_{sample} is the absorbance of the sample containing antioxidant. The antioxidant activity of each sample of extract was expressed as the half maximal inhibitory concentration (IC_{50}) value (mg/mL).

Ferric Reducing Antioxidant Power (FRAP)

The iron-reducing efficacy of sample extracts was evaluated using a modified version of the method developed by Hariyanto et al. (2024). This assay measures the reduction of the colourless Fe^{3+}-tripyridyltriazine complex to a blue Fe^{2+}-tripyridyltriazine complex. The FRAP reagent was prepared by mixing 300 mM acetate buffer, 40 mM of hydrogen chloride (HCl), 10 mM (2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) and 20 mM iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). Standard curves were generated using varying concentrations of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), with all solutions freshly prepared. A reaction mixture consisting of 20 µL of sample solution at different concentrations, 80 µL of distilled water and the FRAP reagent was incubated at 37 °C for 30 minutes. Absorbance was then measured at 593 nm. The difference between the absorbance of the sample and the blank was used to calculate the FRAP value and reduction capacity of the extracts. The FRAP value was expressed in µmol Fe/g.

Nitric Oxide (NO) Radical Scavenging Assay

The inhibitory activity of NO was assessed using the Griess reaction, following the method described by Mfotie Njoya et al. (2017). This approach evaluated the NO scavenging potential of wild ginger extracts and Trolox (used as a standard). Briefly, serial concentrations of the extracts were mixed with sodium nitroprusside ($Na_2[Fe(CN)_5NO]$ and incubated for 20 minutes. After incubation, Griess reagent was added to the mixture, resulting in a pink chromophore, which was measured at 540 nm using a spectrophotometer. The scavenging activity of NO (%) was calculated by using Equation 3:

$$NO\ Radical\ Scavenging\ Assay\ (\%) = \left[\frac{A_{Control} - A_{sample}}{A_{control}}\right] \times 100\% \quad [3]$$

where $A_{control}$ is the absorbance of the NO solution without antioxidant, and A_{sample} is the absorbance of the sample containing antioxidant.

Anti-Diabetic Activity

α-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was carried out following the protocol by Ramadhan et al. (2023). Briefly, 20 µL of the sample was mixed with 30 µL phosphate buffer (pH 6.9), followed by the addition of 50 µL α-glucosidase. After pre-incubation for 10 minutes at 37°C, 45 µL of *p* - nitrophenylglucoside was added to the reaction mixture. Next, 90 µL of sodium carbonate (Na_2CO_3) was added, and the mixture was incubated for 30 minutes at 37°C. Absorbance was then measured at 405 nm. Acarbose and quercetin were used as standard references, and the results were expressed as half-maximal inhibitory concentration (IC_{50}) values.

Data Collection and Statistical Analysis

The experiment was done with three replicates for analysis, and the data generated throughout the experiment were analysed by one-way analysis of variance (ANOVA), whereas the significant differences in means between treatments were separated by using Duncan's Multiple Range test ($p \leq 0.05$) and Pearson's correlation analysis was conducted using IBM SPSS Statistics ver. 30.0.0.0.

RESULTS

Phytocompound Characterisation

In this study, TPC was quantified using the Folin-Ciocalteu colourimetric assay, with gallic acid as the standard. The calibration curve ($y = 2.6769x + 0.4404$, $R^2 = 0.9806$; Supplementary Figure S1) demonstrated strong linearity, and results were expressed as mg gallic acid equivalents (GAE) per gram sample (Table 1). The phenolic content of ethanolic extracts of wild ginger species varied significantly, ranging from 6.53 to 115.77 mg GAE/g DW. Notably, *B. prainiana* and *H. leonurus* displayed the highest TPC in their leaves (115.48 ± 0.36 and 115.77 ± 17.89 mg GAE/g DW, respectively), whereas the lowest phenolic content was observed in the stem of *B. plicata* (3.24 ± 1.47 mg GAE/g DW).

Table 1
Total phenolic content (TPC) for different parts of selected wild ginger species

Species	Total Phenolic Content (mg GAE/g DW)		
	Leaves	Stem	Rhizome
G. patens	$107.23 \pm 3.83_{c}$	$47.71 \pm 17.94_{b}$	$58.21 \pm 9.81_{ab}$
S. klossii	$6.53 \pm 1.03_{a}$	$3.24 \pm 1.47_{a}$	$6.75 \pm 1.31_{a}$
B. plicata	$7.18 \pm 2.90_{a}$	$1.67 \pm 0.49_{a}$	$30.86 \pm 3.01_{ab}$
S. hastilabium	$53.77 \pm 4.04_{b}$	2.38 ± 2.59a	$51.76 \pm 5.42_{ab}$
Amomum sp.	$15.67 \pm 4.13_{a}$	N. D	$45.50 \pm 16.16_{bc}$
H. leonurus	$115.77 \pm 17.89_{c}$	N. D	N. D.
B. prainiana	$115.48 \pm 0.72_{c}$	$26.01 \pm 18.52_{ab}$	$68.43 \pm 20.04_{c}$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-c) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). DW indicates Dry Weight

Table 2
Total flavonoid content (TFC) for different parts of selected wild ginger species

Species	Total Flavonoid Content (mg RE/g DW)		
	Leaves	Stem	Rhizome
G. patens	$9.26 \pm 0.01_{b}$	$1.19 \pm 0.05_{a}$	$2.23 \pm 0.05_{a}$
S. klossii	$10.29 \pm 0.05_{b}$	$3.91 \pm 0.04_{a}$	$4.83 \pm 0.03_{a}$
B. plicata	$11.69 \pm 0.05_{bc}$	$0.81 \pm 0.03_{a}$	$4.33 \pm 0.03_{a}$
S. hastilabium	$14.31 \pm 0.02_{cd}$	$2.24 \pm 0.03_{a}$	$3.27 \pm 0.05_{a}$
Amomum sp.	$14.85 \pm 0.04_{d}$	$0.67 \pm 0.05_{a}$	$3.24 \pm 0.04_{a}$
H. leonurus	$3.07 \pm 2.21_{a}$	$0.89 \pm 0.04_{a}$	$1.16 \pm 0.01_{a}$
B. prainiana	$145.66 \pm 1.13_{e}$	$45.89 \pm 13.23_{b}$	$43.53 \pm 15.88_{b}$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-e) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. DW indicates Dry Weight

TFC was measured using the aluminium chloride colourimetric method with rutin as standard. The assay showed linearity ($y = 0.0021x + 0.0708$, $R^2 = 0.9902$; Supplementary Figure S2) with results expressed as mg rutin equivalents (RE) per gram dry weight sample (Table 2). The analysis revealed flavonoid content among wild ginger species varied significantly, ranging from 0.67 to 145.66 mg RE/g DW. Notably, *B. prainiana* leaves exhibited the highest flavonoid accumulation (145.66 ± 1.13 mg RE/g DW), which represents more than a 200-fold increase compared to the lowest values recorded from *Amomum* sp. stems (0.67 ± 0.05 mg RE/g DW).

Finally, the terpenoid contents in wild ginger species were determined via a vanillin-perchloric acid colourimetric assay standardised against linalool, which produced a

linear calibration (y = 1.526x + 0.4346; R^2 = 0.9982; Supplementary Figure S3). Quantification of TTC revealed that the samples varied significantly between species and plant parts (Table 3). The highest concentration was found in ethanolic extracts of *B. prainiana* leaves containing 0.08 ± 0.002 mg LE/g DW, which was approximately 40-fold greater than the lowest concentration found in *G. patens* and *B. prainiana* stems, which was 0.002 ± 0.005 mg LE/g DW. These significant variations highlighted that terpenoid biosynthesis is species-specific, with *B. prainiana* exhibiting a significantly higher capacity for terpenoid accumulation compared to others. It also reflects the well-documented phenomenon that the distribution of secondary metabolites within plants was often organ-specific, with leaves frequently serving as an important part for the synthesis and storage of bioactive compounds due to their role in defence and environmental interaction (Liu et al., 2022).

Table 3
Total terpenoid content (TTC) for different parts of selected wild ginger species

Species	Total Terpenoid Content (mg LE/g DW)		
	Leaves	Stem	Rhizome
G.patens	$0.07\pm0.02_b$	$0.002\pm0.0005_a$	$0.05\pm0.65_c$
S.klossii	$0.04\pm0.001_a$	$0.02\pm0.001_d$	$0.03\pm0.001_b$
B. plicata	$0.04\pm0.01_a$	$0.02\pm0.001_c$	$0.03\pm0.004_b$
S. hastilabium	$0.04\pm0.003_a$	N. D	$0.03\pm0.04_b$
Amomum sp.	$0.07\pm0.01_b$	N. D	N. D
H. leonurus	$0.03\pm0.001_a$	N. D	N. D
B. prainiana	$0.08\pm0.002_b$	$0.002\pm0.005_a$	$0.07\pm0.01_d$

Note. *Values are presented as mean ± Standard deviation (*n=3*). For columns of each species, values followed by the same letter (a-d) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). DW indicates Dry Weight

Antioxidant Activities

The antioxidant capacity of the ethanolic extracts of wild ginger species varied significantly among species and plant parts (Table 4, Table 5, Table 6, and Table 7). In this study, *S. hastilabium* showed the strongest scavenging activity against both ABTS and NO radicals, with its rhizome being most potent in the ABTS assay (0.03 ± 0.003 mg/mL), while its stem was most potent in the NO assay (2.45 ± 0.881 mg/mL). In *B. prainiana*, the rhizome had the highest DPPH radical scavenging activity (0.02 ± 0.01 mg/mL). Meanwhile, the leaves of *H. leonurus* demonstrated the highest ferric reducing antioxidant power (FRAP) (2.813 ± 0.025 µmol Fe/g). These results indicate that although leaves and rhizome act as major contributors to overall antioxidant capacity, the dominant organ is highly dependent on the specific species and assay type.

Table 4
DPPH antioxidant assay for different parts of wild ginger species

Species	IC50 (mg/mL)		
	Leaves	Stem	Rhizome
G. patens	N.D.	$6.10 \pm 0.36_{b}$	$5.34 \pm 5.14_{b}$
S. klossii	$0.40 \pm 0.17_{a}$	$4.28 \pm 0.75_{ab}$	$3.280 \pm 0.17_{ab}$
B. plicata	$0.46 \pm 0.17_{a}$	$4.94 \pm 1.54_{b}$	$0.05 \pm 0.04_{a}$
S. hastilabium	$0.25 \pm 0.01_{a}$	$0.6 \pm 0.21_{a}$	$0.15 \pm 0.01_{a}$
Amomum sp.	$1.08 \pm 0.07_{b}$	$3.69 \pm 0.68b_{ab}$	$5.29 \pm 1.14_{b}$
H. leonurus	$0.11 \pm 0.07_{a}$	$4.28 \pm 0.75_{ab}$	$0.39 \pm 0.12_{a}$
B. prainiana	$0.02 \pm 0.01_{a}$	$0.37 \pm 0.47_{a}$	$0.21 \pm 0.12_{a}$
Ascorbic acid		0.006 ± 0.01	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For each column of each species, values followed by the same letter (a-d) are not significantly different at $p \leq 0.05$ measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). Ascorbic acid was used as standard

Table 5
ABTS antioxidant assay for different parts of wild ginger species

Species	IC50 (mg/mL)		
	Leaves	Stem	Rhizome
G. patens	$0.09 \pm 0.01_{a}$	$0.45 \pm 0.06_{a}$	$0.38 \pm 0.09_{b}$
S. klossii	$1.58 \pm 0.18_{c}$	$4.46 \pm 0.46_{e}$	$1.54 \pm 0.02_{c}$
B. plicata	$0.73 \pm 0.24_{b}$	$3.50 \pm 0.06_{d}$	$0.39 \pm 0.13_{b}$
S. hastilabium	$0.04 \pm 0.01_{a}$	$0.72 \pm 0.1_{ab}$	$0.03 \pm 0.003_{a}$
Amomum sp.	$2.76 \pm 0.03_{d}$	$2.77 \pm 0.6_{c}$	$4.05 \pm 0.3_{d}$
H. leonurus	$0.04 \pm 0.01_{a}$	$0.81 \pm 0.05_{ab}$	N.D.
B. prainiana	$0.17 \pm 0.03_{a}$	$1.39 \pm 0.7_{b}$	N. D.
Ascorbic acid		0.04 ± 0.00	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-e) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. N. D indicates Not Detected; calculated values below the detection limit of the assay (negative values obtained after calibration curve). Ascorbic acid was used as standard

Table 6
FRAP antioxidant assay for different parts of wild ginger species

Species	FRAP (µmol Fe/g)		
	Leaves	Stem	Rhizome
G.patens	$1.547 \pm 0.03_{e}$	$1.186 \pm 0.02_{f}$	$0.984 \pm 0.02_{c}$
S.klossii	$1.063 \pm 0.03_{d}$	$0.613 \pm 0.004_{c}$	$0.405 \pm 0.03_{a}$
B. plicata	$0.891 \pm 0.02_{c}$	$0.545 \pm 0.02_{b}$	$1.178 \pm 0.02_{d}$

Table 6 (*continued*)

Species	FRAP (µmol Fe/g)		
	Leaves	Stem	Rhizome
S. hastilabium	$0.905 \pm 0.02_{c}$	$0.805 \pm 0.03_{d}$	$1.770 \pm 0.07_{f}$
Amomum sp.	$0.736 \pm 0.03_{b}$	$0.405 \pm 0.03_{a}$	$0.564 \pm 0.04_{b}$
H. leonurus	$2.813 \pm 0.03_{f}$	$1.173 \pm 0.02_{f}$	$1.252 \pm 0.02_{e}$
B. prainiana	$0.474 \pm 0.01_{a}$	$1.134 \pm 0.01_{e}$	$1.008 \pm 0.03_{c}$
Iron (II) sulfate ($FeSO_4$)		1.513 ± 0.01	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-f) are not significantly different at $p \leq 0.05$, *as* measured by one-way ANOVA with Duncan's test. $FeSO_4$ was used as standard

Table 7
Nitric Oxide (NO) antioxidant assay for different parts of wild ginger species

Species	IC50 (µg/mL)		
	Leaves	Stem	Rhizome
G.patens	$7.37 \pm 0.82_{b}$	$14.46 \pm 1.13_{a}$	$36.53 \pm 1.27_{b}$
S.klossii	$15.34 \pm 0.60_{d}$	$24.27 \pm 0.62_{b}$	$14.34 \pm 1.76_{d}$
B. plicata	$22.40 \pm 0.59_{g}$	$33.07 \pm 0.53_{d}$	$38.27 \pm 0.53_{f}$
S. hastilabium	$6.86 \pm 1.29_{f}$	$2.45 \pm 0.88_{a}$	$50.27 \pm 0.33_{a}$
Amomum sp.	$17.38 \pm 0.72_{e}$	$53.15 \pm 0.23_{c}$	$72.43 \pm 0.48_{g}$
H. leonurus	$37.64 \pm 0.69_{c}$	$30.33 \pm 1.75_{e}$	$34.15 \pm 0.78_{e}$
B. prainiana	$8.72 \pm 1.68_{a}$	$21.16 \pm 2.91_{a}$	$19.40 \pm 0.17_{c}$
Trolox		183.14 ± 26.05	

Note. *Values are presented as mean ± Standard Deviation (*n=3*). For columns of each species, values followed by the same letter (a-g) are not significantly different at $p \leq 0.05$, as measured by Duncan's test. Trolox was used as standard

Correlation between Phytochemical Contents and Antioxidant Activities

Pearson correlation analysis (Table 8) revealed significant associations between phenolic content and antioxidant activities. TPC showed a moderate negative correlation with ABTS IC50 values ($r = -0.481$, $p < 0.05$) and a moderate positive correlation with FRAP values ($r = 0.510$, $p < 0.05$), indicating that phenolics significantly contributed to ABTS radical scavenging and ferric-reducing capacities. There were no significant correlations between TPC and either DPPH or NO assays. On the other hand, TFC and TTC did not show significant correlations with any of the antioxidant assays.

Table 8
Pearson's correlation coefficient values (r) between phytochemical and antioxidant activities of wild ginger species

Variable	DPPH	ABTS	FRAP	NO
TPC	r = -0.196	r = -0.481*	r = 0.510*	r =-0.096
TFC	r = 0.266	r = -0.215	r = -0.184	r =-0.329
TTC	r = -0.109	r = 0.273	r =0.018	r =-0.403

Note. *Pearson correlation coefficient values are significant at the $p < 0.05$ level (2-tailed). r refers to the Pearson coefficient

α-Glucosidase Inhibitory Activity

In this study, ethanolic extracts of leaves, stems, and rhizomes of seven wild ginger species from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia were evaluated for α-glucosidase inhibitory activity. The majority of the extracts showed negligible or even negative inhibition, indicating no measurable enzyme suppression at the evaluated concentrations. Only the rhizomes of *B. plicata* and *S. hastilabium* showed detectable inhibition, with IC50 values of 16.77 ± 0.138 µg/mL and 20.26 ± 0.145 µg/mL, respectively (Table 9).

Table 9
α-glucosidase inhibitory assay for different parts of wild ginger species

Species	IC50 (µg/mL)		
	Leaves	Stem	Rhizome
G.patens	NI	NI	NI
S.klossii	NI	NI	NI
B. plicata	NI	NI	16.77 ± 0.138
S. hastilabium	NI	NI	20.26 ± 0.145
Amomum sp.	NI	NI	NI
H. leonurus	NI	NI	NI
B. prainiana	NI	NI	NI
Acarbose		39.85 ± 0.09	
Quercetin		8.36 ± 1.47	

Note. *Values are presented as mean ± Standard Deviation ($n=3$). NI indicates No Inhibition; inhibitory effects were less than 30% at 40 µg/ml. Acarbose and quercetin were used as standards

DISCUSSION

Phytocompound Characterisation

Phenolic compounds are a major class of phytochemicals, exhibiting significant antioxidant activity due to their redox properties, primarily mediated by hydroxyl groups

that facilitate free radical scavenging (Aryal et al., 2019). The reported TPC in leaves may reflect their role in reducing oxidative stress and pathogen defence, which is often caused by phenolic accumulation (Salunke & Koche, 2023). The TPC values in this study were generally lower than those reported for other ginger species. For instance, Fahmi et al. (2024) documented significantly higher TPC in *Etlingera elatior* leaves (483.79 ± 2.57 mg GAE/g) compared to its flowers and stems. On the other hand, a study reported TPC values of 5.95-19.68 mg GAE/g in ethanolic extracts of hybrid and local ginger varieties, while another study observed a range of 9.42-10.09 mg GAE/g in ethanolic ginger extracts (Akullo et al., 2023; Ezez & Tefera, 2021). Large-scale screenings of 24 Zingiberaceae species revealed TPC ranges in 23.80-321.23 mg GAE/g, which indicates Zingiberaceae variability (Generalić Mekinić et al., 2019). These variations may arise from species-specific differences, growth conditions or extraction protocols (Sytar et al., 2018). Despite these variations, our findings suggest that all parts of *B. prainiana* are promising phenolic-rich species among the evaluated wild gingers, warranting further investigation into its bioactive potential.

Flavonoids are another subclass of polyphenolic compounds that are characterised by their benzopyrone backbone and hydroxylated aromatic rings. They have important ecological functions in plants and provide significant health benefits for humans (Nicolescu et al., 2025). As secondary metabolites, they contribute to plant defence against UV radiation, pathogens and oxidative stress. These compounds are becoming increasingly valued for use in pharmaceutical and nutraceutical applications due to their strong antioxidant, anti-inflammatory and anticancer properties. Previous studies revealed that ginger leaves accumulate 2-3 times higher flavonoid content than rhizomes, while another study documented solvent-dependent variations (1.84-2.54 mg QE/g) in local ginger cultivars (Akullo et al., 2023; Ghasemzadeh et al., 2010b). This tissue-specific variation aligns with previous reports where leaves often accumulate higher flavonoid levels than stems, suggesting their functions in protecting photosynthetic tissues and mediating ecological interactions such as UV protection and herbivore deterrence (Davies et al., 2020). *B. prainiana* has a high potential as a source of natural antioxidants due to its exceptional flavonoid concentrations. These findings suggest further investigation into the specific flavonoid subclasses present, optimal extraction protocols and potential synergistic effects with other phytochemicals in this understudied species.

Terpenoids constitute one of the largest and most structurally diverse classes of plant secondary metabolites that mediate essential ecological interactions in plants by serving as chemical defences against herbivores, diseases, and competing vegetation (Das et al., 2022). However, quantitative analyses remain scarce, with most research emphasising rhizomes despite previous studies having often focused on qualitative investigations of the presence or lack of terpenoids in cultivated ginger leaves (Kim et al., 2024; Koo & Gang, 2012).

In this study, *B. prainiana* accumulates terpenoids at concentrations similar to those reported in well-characterised medicinal species such as *K. galanga*, *Z. officinale*, and *C. longa,* which are known for their terpenoid-rich essential oils and related antimicrobial and antioxidant properties (Wang et al., 2023a; Kim et al., 2024; Gu et al., 2024). These results provide the first comprehensive quantification of both foliar and rhizomic terpenoids across wild Zingiberaceae species. This finding also indicates *B. prainiana* as a particularly promising candidate for detailed phytochemical characterisation of specific terpenoid profiles, investigation of biosynthetic pathway regulation and development of standardised extraction protocols for potential antimicrobial applications. The demonstrated variability across wild ginger species highlights the untapped chemical diversity within this taxon and emphasises the need for expanded phytochemical surveys of understudied Zingiberaceae.

Several limitations should be considered when evaluating the phytochemical results. The first limitation is that absolute ethanol was used as the only solvent for extraction, which may underestimate the abundance of lipophilic constituents, particularly non-polar terpenoids and preferentially extracts polar and moderately polar molecules (Azmir et al., 2013; Khaw et al., 2017). The second limitation is that TPC was measured using the vanillin-perchloric acid colourimetric assay, which provides only an estimation of total terpenoid abundance and lacks specificity to any particular terpenoid classes. Previous studies have reported that this assay may also react with certain phenolic compounds, which could influence the accuracy of the measured values (Das et al., 2022). As a result, the total terpenoid contents reported in this study should be interpreted as approximate values rather than absolute concentrations. Future studies using sequential extraction combined with chromatographic techniques such as GC-MS and LC-MS/MS are recommended to achieve comprehensive characterisation of terpenoid contents.

Overall, the leaf part consistently contained higher phenolic, flavonoid and terpenoid levels compared to stems and rhizomes across all the evaluated wild ginger species (leaves > stems and rhizomes). This is because leaves are continuously exposed to environmental stresses such as oxidative stress, UV radiation and herbivores, which require greater production of protective secondary metabolites. Rhizomes served as storage and regenerative organs that exhibited intermediate levels, whereas stems generally had the lowest content due to their structural role. These results highlight the importance of evaluating multiple plant parts rather than focusing solely on rhizomes as commonly done in many Zingiberaceae studies.

Antioxidant Activities

The antioxidant activity reported in this study reflects the general distribution of phytochemicals among different plant parts. Leaves with higher total phenolic and flavonoid contents consistently demonstrated potent radical scavenging activities in DPPH,

ABTS, and nitric oxide assays, supporting the established roles of phenolic compounds as significant contributors to antioxidant defence. Phenolics and flavonoids possess hydroxyl groups that are capable of donating electrons or hydrogen atoms to neutralise reactive oxygen species and inhibit oxidation chain reactions (Aryal et al., 2019; Ulewicz-Magulska & Wesolowski, 2023). On the other hand, rhizomes showed considerably higher ferric-reducing capacities in several species, despite having moderate phenolic levels, suggesting the presence of compounds with stronger reducing potential. Stems generally had weaker antioxidant activities due to their predominantly structural role and lower accumulation of secondary metabolites. Similar organ-specific variations have been reported in cultivated Zingiberaceae species and other medicinal plants, where the genetic background, tissue specialisation, and environmental adaptation affect the antioxidant production and accumulation (Avci et al., 2019; Sinkovič et al., 2024; Yang et al., 2022).

This organ-specific antioxidant variation is strongly correlated with the distribution of phytochemicals present in the same plant parts. The high TPC and TFC accumulation in leaves consistently showed stronger radical scavenging activity in DPPH, ABTS and NO, supporting the well-established role of phenolics and flavonoids as main antioxidant compounds. Meanwhile, rhizomes with intermediate phenolic levels showed significant reducing power (FRAP) likely due to accumulated storage metabolites and defence. On the other hand, stems primarily serve as structural organs and contain the lowest phytochemical concentrations and correspondingly weaker antioxidant potential (Mahmudati et al., 2023). These variations are commonly attributed to species-specific genetic factors that affect the biosynthesis and accumulation of secondary metabolites, as reported in recent phytochemical studies (Avci et al., 2019; Sinkovič et al., 2024; Yang et al., 2022).

The variations observed among antioxidant assays highlight the importance of using multiple analytical approaches to fully assess antioxidant potential. DPPH and ABTS assays mainly measure radical scavenging through hydrogen atom transfer and single-electron transfer mechanisms, while FRAP evaluates ferric ion reducing capacity (Ulewicz-Magulska & Wesolowski, 2023). Therefore, the same extract may show different antioxidant capabilities depending on the underlying reaction mechanism and the chemical nature of the bioactive constituents present. For instance, the superior ABTS radical scavenging activity exhibited by *S. hastilabium* rhizomes and the strong ferric reducing capacity observed in *H. leonurus* leaves suggest that various metabolite classes contribute to antioxidant activity through different mechanisms. Similar assay-dependent variations have been reported in *Z. officinale* and *C. longa*, in which radical scavenging and reducing capacities differed among plant organs and extraction systems (Ghasemzadeh et al., 2010a; Ulewicz-Magulska & Wesolowski, 2023). These findings highlight that no single antioxidant assay is sufficient to fully characterise the antioxidant potential of medicinal plants.

Overall, leaves emerged as the principal reservoirs of antioxidant phytochemicals in most of the investigated species, followed by rhizomes, whereas stems generally exhibited the lowest antioxidant activities. This pattern reflects the physiological roles of the different organs. Leaves are metabolically active tissues that are continuously exposed to ultraviolet radiation, herbivores, and oxidative stress, thereby requiring higher concentrations of protective secondary metabolites. Rhizomes serve as storage and regenerative organs and thus maintain moderate concentrations of defence compounds, whereas stems mainly provide structural support and consequently invest less in secondary metabolite biosynthesis (Mahmudati et al., 2023). These findings highlight the importance of evaluating multiple plant organs rather than focusing exclusively on rhizomes, as commonly practised in many Zingiberaceae studies. Furthermore, the strong antioxidant activities observed, particularly in leaf extracts, suggest that wild Malaysian gingers represent promising and sustainable sources of natural antioxidants for nutraceutical and pharmaceutical applications.

Correlation Between Phytochemical Contents and Antioxidant Activities

Phenolic compounds appeared to be the main contributors to antioxidant activity in the investigated wild ginger species. Significant correlations between TPC and both ABTS and FRAP assays indicate that phenolic constituents are largely responsible for radical scavenging and reducing capacities. Similar relationships have been reported in *Zingiber officinale* and *Curcuma longa*, where phenolic compounds were identified as major determinants of antioxidant performance (Ghasemzadeh et al., 2010a; Ulewicz-Magulska & Wesolowski, 2023). Differences in radical chemistry and the selectivity of specific oxidising species may be the cause for the absence of significant correlations between TPC and DPPH or nitric oxide assays (Nicolescu et al., 2025).

Similarly, the lack of significant correlations between TFC and TTC suggests that antioxidant potential is not always determined by total flavonoid and terpenoid contents alone. Previous studies have shown that the chemical structures of individual compounds have a significant influence on antioxidant capabilities rather than their total concentration. The ability of flavonoids and terpenoids to scavenge radicals is significantly influenced by structural features such as the number and position of hydroxyl groups, conjugated double bonds, and glycosylation patterns (Lu et al., 2024; Wang et al., 2023a). Therefore, total phytochemical contents should be interpreted cautiously because they may not accurately reflect the biological activities of specific metabolites. Future investigations using chromatographic and metabolomic approaches such as LC-MS/MS are required to identify the compounds responsible for the observed antioxidant activities.

Overall, the present findings demonstrate that phenolic compounds play a central role in the antioxidant properties of wild Malaysian gingers, particularly in leaf tissues, and further support their potential as valuable natural sources of antioxidant compounds.

α-Glucosidase Inhibitory Activity

α-Glucosidase is a key enzyme involved in postprandial carbohydrate digestion, and its inhibition is an established therapeutic strategy for controlling hyperglycaemia in type 2 diabetes mellitus (Han et al., 2025). Although acarbose is widely used as a clinical α-glucosidase inhibitor, its use is often associated with gastrointestinal side effects, thereby stimulating interest in plant-derived alternatives with fewer adverse effects (Akmal et al., 2024). The α-glucosidase inhibitory activities observed in this study were comparable to those reported for several cultivated Zingiberaceae species. For instance, methanolic rhizome extracts of *Z. officinale* exhibited an IC50 value of 18.5 µg/mL (Maniam et al., 2024), while *Aframomum melegueta* fruits rich in 6-gingerol and oleanolic acid demonstrated potent inhibitory activity with an IC50 value of 17.35 ± 0.88 µM (Mohammed et al., 2017). A similar significant inhibitory effect was shown by the rhizome extracts of *B. plicata* and *S. hastilabium,* suggesting that these wild species may represent promising sources of natural antidiabetic agents.

Interestingly, leaves and stems showed negligible α-glucosidase inhibitory activity despite possessing higher total phenolic and flavonoid contents. This observation suggests that total phytochemical abundance alone does not necessarily translate into enzyme inhibition. Previous studies have shown that inhibitory activity is mainly dependent on the presence of specific metabolites and their structural characteristics rather than overall phenolic or flavonoid concentrations (Wu et al., 2023). Diarylheptanoids, phenylpropanoids, flavonoids, and terpenoids are examples of bioactive compounds that have been reported to inhibit α-glucosidase through different mechanisms, highlighting the significance of compound composition rather than bulk metabolite content (Han et al., 2025). Therefore, the absence of measurable inhibition in most species and organs may reflect either the absence of these active constituents or their low abundance in the ethanolic extracts.

Although the active compounds responsible for the potent inhibitory activities observed in *B. plicata* and *S. hastilabium* rhizomes remain unknown, the present findings indicate that specific metabolites are likely involved. Consequently, future studies employing bioassay-guided fractionation and metabolomic approaches such as LC-MS/MS are required to identify and characterise the compounds responsible for the observed α-glucosidase inhibitory activity. Such investigations would facilitate a better understanding of the antidiabetic potential of these underexplored wild gingers and may contribute to the discovery of novel natural inhibitors.

Several limitations should be considered when interpreting the antidiabetic findings of this study. The α-glucosidase inhibitory activity was evaluated using a single-endpoint assay without detailed kinetic characterisation. Therefore, the mode of inhibition and enzyme-substrate interactions remain unclear. Enzyme kinetic analyses to differentiate competitive, non-competitive, and mixed modes of inhibition could provide deeper insights

into the mechanisms underlying enzyme suppression (Lam et al., 2024; Lu et al., 2023). Accordingly, future studies should incorporate enzyme kinetic analyses with bioassay-guided fractionation and metabolomic profiling to elucidate the active compounds and their mechanisms of action.

CONCLUSION

In conclusion, this study represents the first comprehensive comparative study that evaluates phytochemical composition, antioxidant activities, and α-glucosidase inhibitory potential between different plant parts (leaves, stems, and rhizomes) of seven wild Zingiberaceae species that were collected from Panti Forest Reserve and Endau-Rompin National Park, Johor, Malaysia. The findings showed significant variations in phytochemical accumulation and biological activities across different plant parts. In general, leaves contained higher total phenolic, flavonoid, and terpenoid contents and exhibited superior antioxidant activities, highlighting their importance as metabolically active tissues rich in protective secondary metabolites. The antioxidant properties of these wild ginger species are mainly attributed to phenolic compounds, as demonstrated by significant correlations between total phenolic content and ABTS and FRAP assays.

B. prainiana demonstrated the highest phytochemical contents among the studied species, whereas *H. leonurus* and *S. hastilabium* showed exceptional antioxidant capacities in selected assays. The rhizomes of *B. plicata* and *S. hastilabium* showed measurable enzyme inhibition with IC50 values lower than that of acarbose, suggesting the presence of potent antidiabetic constituents. These findings indicate that enzyme inhibition is governed by specific metabolites rather than total phytochemical abundance alone.

Overall, the present study highlights the untapped phytochemical diversity and therapeutic potential of wild Malaysian Zingiberaceae, particularly their leaves, which may serve as sustainable alternative sources of natural antioxidants for nutraceutical and pharmaceutical applications. Furthermore, the promising α-glucosidase inhibitory activities observed in *B. plicata* and *S. hastilabium* rhizomes warrant further investigation through bioassay-guided fractionation, enzyme kinetic studies, and metabolomic approaches such as LC-MS/MS and GC-MS to identify the compounds responsible for their biological activities. Expanding phytochemical and pharmacological investigations to other underexplored wild Zingiberaceae species may facilitate the discovery of novel bioactive compounds while supporting the sustainable utilisation and conservation of Malaysia's rich botanical resources.

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SUPPLEMENTARY DATA

Raw Absorbance Values for Total Phenolic Content of Wild Ginger Species

Table S1
Raw absorbance values of gallic acid used for total phenolic content standard curve

Concentration (mg/ml)	Absorbance			Blank Sample
	1	2	3	
1.000	3.206	3.0564	3.1888	3.1455
0.500	1.6486	1.4124	1.7845	0.3108
0.250	1.6771	1.0412	1.247	1.1871
0.125	0.8332	0.8214	0.7125	0.706
0.063	0.6025	0.4462	0.4881	0.4924

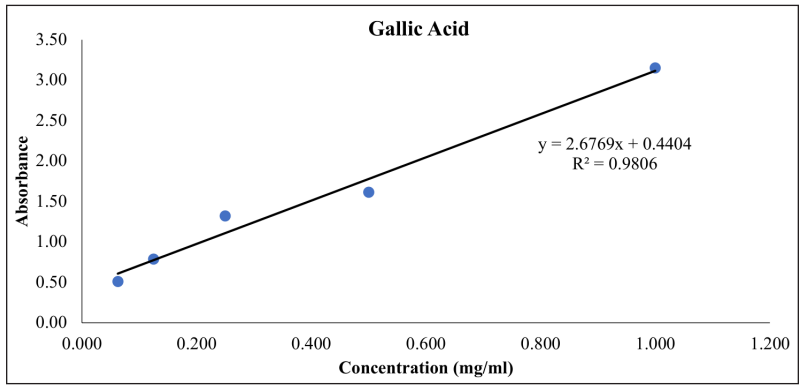


Figure S1. The standard curve of gallic acid

Standard Rutin for Total Flavonoid Content

Table S2
Raw absorbance values of rutin used for total flavonoid content standard curve

Concentration (mg/ml)	Absorbance			Blank Sample
	1	2	3	
0.250	1.2415	1.2452	1.2761	1.1523
0.125	0.7349	0.7277	0.762	0.5028
0.063	0.4427	0.4284	0.4256	0.4176
0.032	0.2511	0.2365	0.2422	0.2314
0.016	0.19	0.2034	0.2102	0.1587

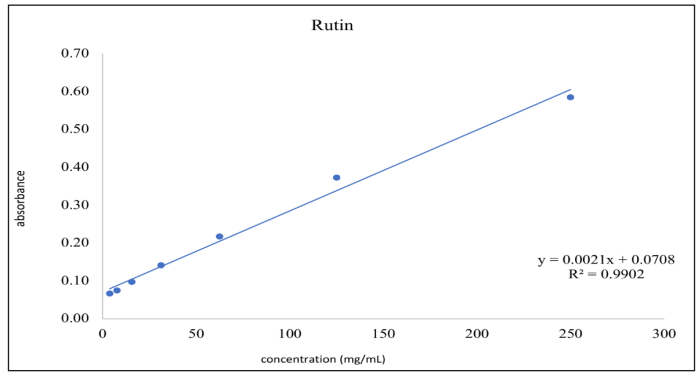


Figure S2. The standard curve of Rutin

Standard Linalool for Total Terpenoid Content

Table S3
Raw absorbance of linalool values used for total terpenoid content standard curve

Concentration (mg/ml)	Absorbance
1.00	1.0705
0.80	0.812
0.50	0.3407
0.40	0.1675
0.30	0.014

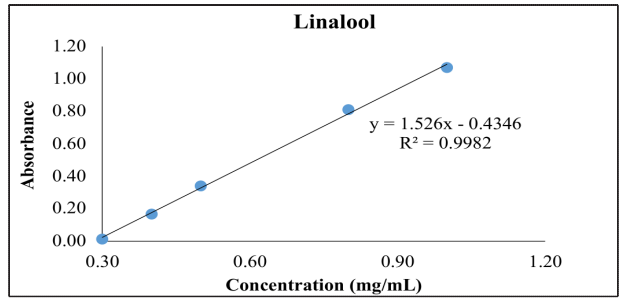


Figure S3. The standard curve of Linalool

Standard Ascorbic acid for 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Table S4
Raw absorbance of ascorbic acid values used for DPPH standard curve

Concentration (mg/mL)	Absorbance			Blank Sample
	1	2	3	
0.0125	0.1083	0.1303	0.1176	0.0384
0.0063	0.151	0.148	0.1449	0.0388
0.0031	0.1636	0.1765	0.1657	0.0402
0.0016	0.1743	0.18	0.1783	0.0408

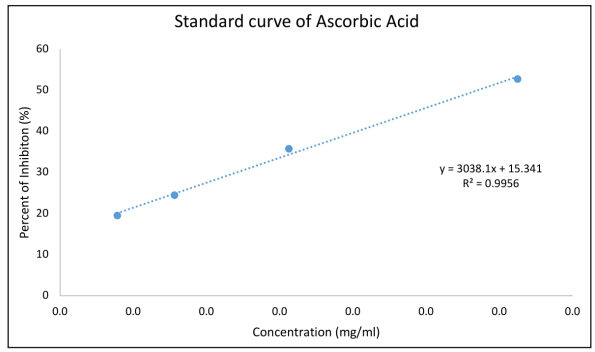


Figure S4. Standard calibration curve of ascorbic acid for DPPH assay

Standard Ascorbic acid for 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Assay

Table S5
Raw absorbance of standard ascorbic acid values used for ABTS

Concentration (mg/mL)	Absorbance			Blank Sample
	1	2	3	
0.100	0.0392	0.0405	0.0395	0.0374
0.050	0.3608	0.4229	0.4261	0.0505
0.025	0.631	0.6656	0.6883	0.0379
0.013	0.7768	0.807	0.7902	0.037
0.006	0.8414	0.8697	0.852	0.036
0.003	0.8806	0.8947	0.8864	0.0374

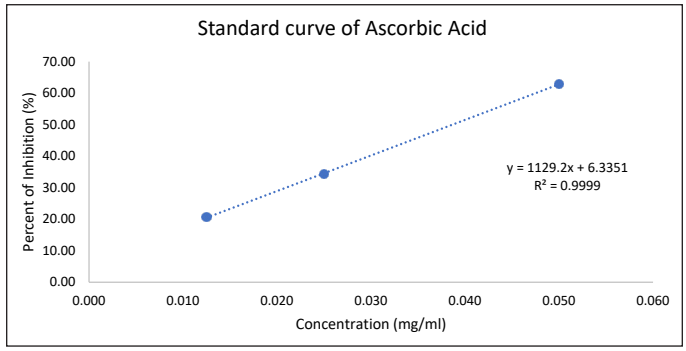


Figure S5. Standard calibration curve of ascorbic acid for ABTS assay

Standard Iron (II) sulphate for Ferric Reducing Antioxidant Power (FRAP)

Table S6
Raw absorbance of standard $FeSO_4$

Concentration (ppm)	Absorbance			Blank Sample
	1	2	3	
500	1.6236	1.6142	1.6021	0.0997
400	1.3123	1.4784	1.3550	0.0978
300	1.1154	1.0251	1.1950	0.0960
200	0.9189	0.8397	0.7115	0.1046
100	0.5101	0.5005	0.4836	0.1024
500	1.6236	1.6142	1.6021	0.0997

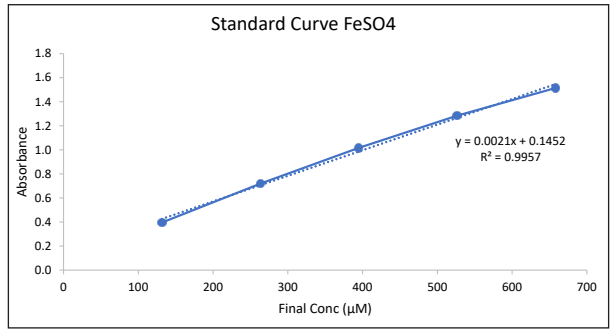


Figure S6. Standard calibration curve of FeSO4 for FRAP assay

Standard Trolox for Nitric Oxide (NO) Radical Scavenging Assay

Table S7
Raw absorbance of standard Trolox

Concentration (ppm)	Absorbance			Blank Sample
	1	2	3	
400	0.5596	0.5504	0.5579	0.0997
200	0.2422	0.2322	0.2331	0.0978
100	0.2814	0.2807	0.2734	0.0960
50	0.3027	0.3081	0.3184	0.1046
25	0.3217	0.3221	0.3229	0.1024
13	0.3376	0.3350	0.3246	0.0997

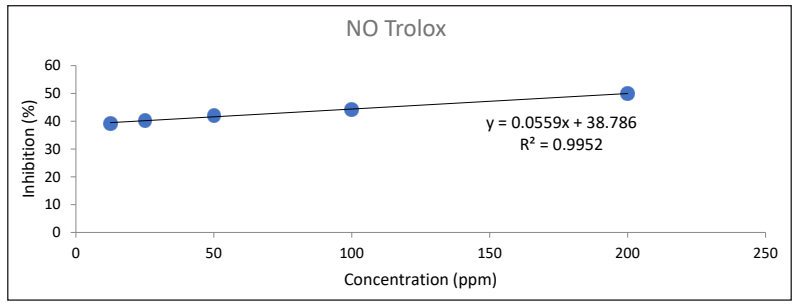


Figure S7. Standard calibration curve of Trolox for NO assay

Statistical Analysis

Total Phenolic Content

Table S8
ANOVA test total phenolic content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	6	0.000	49.289	<0.001
Within Groups	0.000	14	0.000		
Total	0.000	20			

Table S9
Duncan's test total phenolic content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05		
			1	2	3
Duncan[a]	*S. klossii*	3	0.000833		
	B. plicata	3	0.000867		
	Amomum sp.	3	0.001167		
	S. hastilabium	3		0.002533	
	G. patens	3			0.004433
	B. prainiana	3			0.004733
	H. leonurus	3			0.004767
	Sig.		.411	1.000	0.411

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S10
ANOVA test total phenolic content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.000	6	0.000	3.662	0.021
Within Groups	0.000	14	0.000		
Total	0.000	20			

Table S11
Duncan's test total phenolic content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*Amomum* sp.	3	0.000367	
	H. leonurus	3	0.000533	
	B. plicata	3	0.000667	
	S. klossii	3	0.000700	
	S. hastilabium	3	0.000700	
	B. prainiana	3	0.001533	0.001533
	G. patens	3		0.002333
	Sig.		0.063	0.146

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S12
ANOVA test total phenolic content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.657	5	0.531	40.352	<0.001
Within Groups	0.158	12	0.013		
Total	2.815	17			

Table S13
Duncan's test total phenolic content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05		
			1	2	3
Duncan[a]	S. klossii	3	0.030804		
	Amomum sp.	3	0.204481		
	S. hastilabium	3	0.215143		
	B. plicata	3	0.231199		
	G. patens	3		0.607730	
	B. prainiana	3			1.178491
	Sig.		0.070	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Total Flavonoid Content

Table S14
ANOVA test total flavonoid content for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	188375.893	6	31395.982	2644.961	<0.001
Within Groups	166.182	14	11.870		
Total	188542.074	20			

Table S15
Duncan's test total flavonoid content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*H. leonurus*	3	5.09333				
	G. patens	3		17.46000			
	S. klossii	3		19.53333	19.53333		
	B. plicata	3			24.14667	24.14667	
	S. hastilabium	3				27.56000	
	Amomum sp.	3				28.64333	
	B. prainiana	3					290.25333
	Sig.		1.000	0.473	0.123	0.151	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S16
ANOVA test total flavonoid content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	54369178.234	6	9061529.706	67.922	<0.001
Within Groups	1867748.318	14	133410.594		
Total	56236926.552	20			

Table S17
Duncan's test total flavonoid content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*Amomum* sp.	3	4.5238	
	H. leonurus	3	7.4603	
	B. plicata	3	8.4788	
	G. patens	3	16.6601	
	S.klossii	3	63.5913	
	S. hastilabium	3	64.4444	
	B. prainiana	3		4625.2230
	Sig.		0.858	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S18
ANOVA test total flavonoid content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11849134.732	6	1974855.789	122.540	<0.001
Within Groups	225624.247	14	16116.018		
Total	12074758.979	20			

Table S19
Duncan's test total flavonoid content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*H. Leonurus*	3	3.9667	
	S. hastilabium	3	23.0200	
	Amomum sp.	3	28.4333	
	G. patens	3	35.3100	
	S. klossii	3	35.7333	
	B. plicata	3	55.4967	
	B. prainiana	3		2176.5600
	Sig.		0.660	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Total Terpenoid Content

Table S20
ANOVA test total terpenoid content for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.007	6	0.001	32.571	<0.001
Within Groups	0.000	14	0.000		
Total	0.007	20			

Table S21
Duncan's test total terpenoid content for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*H.leonurus*	3	0.0300	
	S. klossii	3	0.0400	
	B.plicata	3	0.0400	
	S. hastilabium	3	0.0400	
	G. patens	3		0.0700
	Amomum sp.	3		0.0700
	B. prainiana	3		0.0767
	Sig.		0.069	0.200

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S22
ANOVA test total terpenoid content for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.002	6	0.000	71.000	<0.001
Within Groups	0.000	14	0.000		
Total	0.002	20			

Table S23
Duncan's test total terpenoid content for stem wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*G. patens*	3	0.0000			
	H. leonurus	3	0.0000			
	B. prainiana	3	0.0000			
	S. hastilabium	3		0.0100		
	Amomum sp.	3		0.0100		
	B.plicata	3			0.0200	
	S. klossii	3				0.0267
	Sig.		1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S24
ANOVA test total terpenoid content for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.009	6	0.002	29.030	<0.001
Within Groups	0.001	14	0.000		
Total	0.010	20			

Table S25
Duncan's test total terpenoid content for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*H. leonurus*	3	0.0000			
	Amomum sp.	3	0.0100			
	S. klossii	3		0.0300		
	S. hastilabium	3		0.0300		
	B. plicata	3		0.0333		
	G. patens	3			0.0500	
	B. prainiana	3				0.0667
	Sig.		0.113	0.601	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

DPPH Radical Scavenging Assay

Table S26
ANOVA test DPPH assay for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	79.894	6	13.316	28.094	<0.001
Within Groups	6.636	14	0.474		
Total	86.529	20			

Table S27
Duncan's test DPPH for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*B. prainiana*	3	0.0233	
	H. leonurus	3	0.1100	
	S. hastilabium	3	0.2500	
	S. klossii	3	0.4033	
	B. plicata	3	0.4600	
	Amomum sp.	3	1.0825	
	G. patens	3		5.8867
	Sig.		0.112	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S28
ANOVA test DPPH assay for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	76.974	6	12.828	2.718	0.58
Within Groups	66.071	14	4.719		
Total	143.041	20			

Table S29
Duncan's test DPPH for stem wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	*B. prainiana*	3	0.3733	
	S. hastilabium	3	0.6000	
	S. klossii	3	1.7200	1.7200
	Amomum sp.	3	3.6891	3.6891
	H. leonurus	3	4.2767	4.2767
	B. plicata	3	1.0825	4.9400
	G. patens	3		5.3433
	Sig.		0.065	0.084

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S30
ANOVA test DPPH assay for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	109.757	6	18.293	4.636	0.009
Within Groups	55.239	14	3.946		
Total	164.996	20			

Table S31
Duncan's test DPPH for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05	
			1	2
Duncan[a]	B. plicata	3	.0467	
	S. hastilabium	3	.1467	
	B. prainiana	3	.2100	
	H. leonurus	3	.3933	
	S. klossii	3	3.2767	3.2767
	Amomum sp.	3		5.2883
	G. patens	3		5.3433
	Sig.		.092	.246

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

ABTS Radical Scavenging Assay

Table S32
ANOVA test ABTS assay for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.504	6	3.251	253.113	<0.001
Within Groups	0.180	14	0.013		
Total	19.684	20			

Table S33
Duncan's test ABTS for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	S. hastilabium	3	0.0367			
	H. leonurus	3	0.0367			
	G. patens	3	0.0867			
	B. prainiana	3	0.1700			
	B. plicata	3		0.7333		
	S. klossii	3			1.5767	
	Amomum sp.	3				2.7567
	Sig.		0.204	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S34
ANOVA test ABTS assay for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44.197	6	7.366	50.590	<0.001
Within Groups	2.038	14	0.146		
Total	46.235	20			

Table S35
Duncan's test ABTS for stem wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*G. patens*	3	0.4500				
	S. hastilabium	3	0.7167	0.7167			
	H. leonurus	3	0.8100	0.8100			
	B. prainiana	3		1.3900			
	Amomum sp.	3			2.7667		
	B. plicata	3				3.4967	
	S. klossii	3					4.4633
	Sig.		0.291	0.058	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S36
ANOVA test ABTS assay for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.738	6	6.623	356.992	<0.001
Within Groups	0.260	14	0.019		
Total	39.998	20			

Table S37
Duncan's test ABTS for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05			
			1	2	3	4
Duncan[a]	*H. leonurus*	3	0.0000			
	B. prainiana	3	0.0000			
	S. hastilabium	3	0.0300			
	G. patens	3		0.3767		
	B. plicata	3		0.3900		
	S. klossii	3			1.5433	
	Amomum sp.	3				4.0500
	Sig.		0.802	0.906	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Ferric Reducing Antioxidant Power (FRAP)

Table S38
ANOVA test (FRAP) for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11.158	6	1.860	3446.505	<0.001
Within Groups	0.008	14	0.001		
Total	11.165	20			

Table S39
Duncan's test (FRAP) for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*B. prainiana*	3	0.437767					
	Amomum sp.	3		0.736367				
	plicata	3			0.890800			
	S. hastitalibum	3			0.905400			
	S. klossii	3				1.063500		
	G. patens	3					1.547000	
	H. leonurus	3						2.813033
	Sig.		1.000	1.000	0.454	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S40
ANOVA test (FRAP) for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.937	6	0.323	919.887	<0.001
Within Groups	0.005	14	0.000		
Total	1.942	20			

Table S41
Duncan's test for stem wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*Amomum* sp.	3	0.404567					
	B. plicata	3		0.545067				
	S. klossii	3			0.612567			
	S. hastilabium	3				0.805400		
	B. prainiana	3					1.133667	
	H. leonurus	3						1.172700
	G. patens	3						1.185567
	Sig.		1.000	1.000	1.000	1.000	0.052	

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S42
ANOVA test (FRAP) for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.517	6	0.586	534.769	<0.001
Within Groups	0.015	14	0.001		
Total	3.532	20			

Table S43
Duncan's test (FRAP) for stem wild ginger species

	Species	N	Subset for Alpha = 0.05					
			1	2	3	4	5	6
Duncan[a]	*S. klossii*	3	0.452867					
	Amomum sp.	3		0.563967				
	G. patens	3			0.983633			
	B. prainiana	3			1.007933			
	B. plicata	3				1.178100		
	H. leonurus	3					1.252100	
	S. hastilabium	3						1.770300
	Sig.		1.000	1.000	.384	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Nitric Oxide (NO) Antioxidant Assay

Table S44
ANOVA test (NO) for leaves of wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5498.219	6	916.370	1097.742	<0.001
Within Groups	11.687	14	0.835		
Total	5509.906	20			

Table S45
Duncan's test (NO) for leaves of wild ginger species

	Species	N	Subset for Alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a]	*B. prainiana*	3	.000000						
	G. patens	3		14.336233					
	H. leonurus	3			19.398267				
	S. klossii	3				34.148167			
	Amomum sp.	3					36.532533		
	S. hastilabium	3						38.270033	
	B. plicata	3							51.921767
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S46
ANOVA test (NO) for stem wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2161.730	6	360.288	313.485	<0.001
Within Groups	16.090	14	1.149		
Total	2177.820	20			

Table S47
Duncan's test (NO) for stem wild ginger species

	Species	N	Subset for Alpha = 0.05				
			1	2	3	4	5
Duncan[a]	*S. hastilabium*	3	6.859722				
	G. patens	3	7.371192				
	B. prainiana	3	8.717563				
	S. klossii	3		15.336593			
	Amomum sp.	3			17.374953		
	B. plicata	3				22.399398	
	H. leonurus	3					37.638210
	Sig.		0.062	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

Table S48
ANOVA test (NO) for rhizome wild ginger species

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4680.847	6	780.141	364.192	<.001
Within Groups	29.990	14	2.142		
Total	4710.836	20			

Table S49
Duncan's test (NO) for rhizome wild ginger species

	Species	N	Subset for Alpha = 0.05						
			1	2	3	4	5	6	7
Duncan[a]	*S. hastilabium*	3	2.452050						
	G. patens	3		14.464084					
	B. prainiana	3			21.158330				
	S. klossii	3				24.264761			
	H. leonurus	3					30.327191		
	B. plicata	3						33.065062	
	Amomum sp.	3							53.899962
	Sig.		1.000	1.000	1.000	1.000	1.000	1.000	1.000

Note. Means for groups in homogeneous subsets are displayed
a. Uses Harmonic Mean Sample Size = 3.000

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Effects of Legume-Enriched *Moina micrura* on the Growth, Survival, and Nutritional Composition of Giant Freshwater Prawn (*Macrobrachium rosenbergii*) Post-larvae

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ABSTRACT

Moina micrura, a type of freshwater zooplankton known as water fleas commonly employed as live feed for fish in aquaculture and must be nutritionally enriched to provide the best nutrients for crustacean larvae's development and survival. This study aims to improve the growth, reproduction, and nutritional value of *M. micrura* and to analyse the effects of utilising different types of enriched *M. micrura* on protein, lipid, and growth of giant freshwater prawn *Macrobrachium rosenbergii*. The effectiveness of enriched *M. micrura* on *M. rosenbergii* post-larvae was evaluated using three types of legume-based enrichment (soybean, mung bean, and sesame seed) and yeast as a control for triplicates of each. The growth, survival, protein, and lipid compositions of *M. rosenbergii* post-larvae were determined after 30 days to compare the efficacy of each treatment. The results showed that *M. micrura* fed with soybean had the highest survival rate and specific population

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growth rate, with values of 95.00 ± 4.9% and 0.059 ± 0.001%, respectively. A higher specific growth rate of *M. rosenbergii* was observed when fed with *M. micrura* enriched with soybean (5.73 ± 0.21%), and the lowest was when fed with *M. micrura* enriched with mung bean (3.84 ± 0.23%). Soybean enrichment proved to be the most effective among the different enrichments utilised, particularly in the assessment of the proximate analysis of *M. rosenbergii* post-larvae. The mean values for protein and lipid content in *M. rosenbergii* were determined to be 69.20 ± 0.77% and 7.52 ± 0.55%, respectively. The results indicated that it is highly recommended to use *M. micrura* enriched with soybean to feed *M. rosenbergii* post-larvae.

Keywords: Enrichment, growth, legumes, *Moina micrura*, *Macrobrachium rosenbergii*, nutrients

INTRODUCTION

Moina sp., a type of freshwater zooplankton commonly known as water fleas, is a small species of cladoceran crustacean (Joshue et al., 2022; Othman et al., 2024). A diverse array of zooplankton species has been commonly employed as live feed for fish in aquaculture, with Moina being notable due to their diminutive size and erratic motions, which appeal to larval fish (Lomartire et al., 2021). *Moina* sp. possess significant potential as a live food source for larval fish and crustaceans (Islam et al., 2017). *Moina micrura* is particularly suitable as an early feed due to its small size, quick growth, high rate of reproduction, ease of culture, high content of digestive enzymes, and simplicity in managing their nutritional value. Nevertheless, *M. micrura* must be supplemented with highly nutritious feed prior to being provided to fish fry and shrimp larvae because of their nutritional variability. Singh et al. (2019) reported that *Moina* sp. lacks essential nutrients necessary for the growth and survival of predatory fish and crustacean larvae, particularly in n-3 highly unsaturated fatty acids, such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Therefore, it is necessary to improve the nutritional quality by enriching zooplankton, including *Moina* sp, through pre-feeding (Das et al., 2007).

The post-larvae (PL) of giant freshwater prawn *Macrobrachium rosenbergii* depend significantly on dietary protein and lipids for growth; however, Moina alone may be insufficient in supplying these nutrients adequately. Protein is a crucial macronutrient essential for tissue development and moulting; its deficiency may result in delayed development and increased mortality rates. Insufficient protein also can result in inadequate muscle development and prolonged moulting cycles, heightening vulnerability to predation and cannibalism. Lipids are essential for energy provision and metabolic functions. Despite the presence of lipids in Moina, the total lipid content may be insufficient to satisfy the energy requirements of *M. rosenbergii* post-larvae, especially in intensive culture systems with high energy demands. Insufficient lipid intake may cause post-larvae to allocate protein for energy metabolism rather than growth, leading to diminished feed efficiency and slower weight gain (Chuphal et al., 2021).

Alternative plant-based feed sources, including soybean (*Glycine max*), mung bean (*Vigna radiata*), and sesame seed (*Sesamum indicum*), present promising nutritional profiles that may improve zooplankton culture. Soybean is known for its high protein content, comprising roughly 35-40% of its dry weight (Modgil et al., 2020). This protein is abundant in essential amino acids, including arginine and glutamine, which are essential for zooplankton development and enzymatic activities. Mung bean provides a moderate protein content of around 20-25%, featuring a balanced amino acid profile that facilitates zooplankton growth and reproduction. Sesame seed is distinguished by its high lipid content (~50%), featuring substantial amounts of polyunsaturated fatty acids (PUFAs) like linoleic acid, which are crucial for zooplankton reproductive success and larval development (Thiruvasagam et al., 2024). Sesame seed comprises approximately 18-25% protein, though its amino acid profile exhibits reduced lysine levels in comparison to soybean and mung bean.

This study aims to enhance the growth, reproduction, and nutritional quality of *M. micrura* by enriching it with three legume-based diets (soybean, mung bean, sesame seed) alongside yeast as a control, and to evaluate the nutritional transfer effects of enriched *M. micrura* on the protein content, lipid profile, and growth performance of *M. rosenbergii* post-larvae.

MATERIALS AND METHOD

Experimental Setup

M. micrura was sampled from Tasik Kenyir, Kuala Berang, Terengganu, Malaysia. Upon collection, the live samples were rinsed with dechlorinated freshwater (1µm) to prevent contamination and were transported directly to the laboratory. Cladocerans were isolated and classified based on their morphological characteristics. Different species of Moina were identified by studying their body shape and segments of the antenna (Bledzki & Rybak, 2016; Patil et al., 2007; Shiel, 1995; Yong & Yule, 2004).

The study was conducted at the Hatchery of the Faculty of Fisheries and Food Science (FPSM), located at Universiti Malaysia Terengganu, for 30 days. The feeding regimen for *M. micrura* involved a once-daily administration, with a concentration of 500mgL^{-1}, as detailed by Suhaimi et al. (2022a) and Naman et al. (2021). The experimental design consisted of four different dietary treatments with soybean (*Glycine max*), mung bean (*Vigna radiata*), sesame seed (*Sesamum indicum*) and yeast as a control. Each condition was tested in triplicate (4 tanks × 3 replicates).

To assess the growth of population density, *M. micrura* was reared in 25L aquarium tanks, with an initial stocking density of 40 individuals per mL. The feed was prepared fresh daily by weighing powdered feeds, dissolving them in dechlorinated freshwater, and filtering the solution through a 50µm mesh. A feed concentration of 500 mgL^{-1} was then

introduced into the culture tanks (Khoo et al., 2013; Suhaimi et al., 2022b). Water quality was maintained by replacing 50% of the water every two days, with a temperature of 25-27 °C and a pH of 7.5 (Islam et al., 2017; Suhaimi et al., 2022b).

For this study, post-larvae of *M. rosenbergii* PL-5 were used. Upon their arrival, a systematic acclimatisation process was implemented for the post-larvae prior to their stocking into individual aquariums. The experimental design incorporated three replicates for each treatment, including the control, resulting in a comprehensive set of 12 replicates. The cultivation of *M. rosenbergii* post-larvae comprised a total of 12 replicates, for three treatments and one control, where each tank consisted of a total of 100 post-larvae.

The post-larvae received twice-daily feedings of enriched *M. micrura.* Before each feeding session, the enriched *M. micrura* was extracted from the treatment aquarium, washed, sieved and weighed. To enhance nutritional value, *M. micrura* underwent a 6-hour enrichment process within the treatment tank before each post-larvae feeding, following the methodology outlined by Fereidouni et al. (2013). Aeration systems were installed in all aquariums to ensure optimal cultured conditions. Water exchange was done for 50-70% every week, and water temperature (26-27 °C), pH (3-5), salinity (0 ppt), and dissolved oxygen were maintained throughout the experiment.

Diet Preparation and Enrichment of *Moina micrura*

In this study, three types of legume-based treatments and one control treatment were utilised. The three types of legumes (soybean, sesame seed, and mung bean) were washed with clean water to eliminate any foreign particles. Following the thorough cleaning, the legumes underwent a drying process inside the oven at a temperature of 50 °C to eliminate any remaining moisture while preserving their original nutritional properties. Once the legumes were completely dry, they were ground using a grinder to form a fine powdery texture. These powdered legumes were then used to enrich *M. micrura* by mixing them with dechlorinated freshwater until fully dissolved. The feed was sieved through a 50µm mesh (Khoo et al., 2013) prior to being introduced into the culture tank with 4000 individuals per litre of Moina (Rasdi et al., 2021). *M. micrura* was fed the diets once daily at a concentration of 500mgL^{-1}.

Protein and Lipid Analysis

The analysis of protein and lipid was conducted on diet treatments, enriched *M. micrura* and *M. rosenbergii* post-larvae at the Laboratory of General Analysis and Quality at the Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu. *M. micrura* was enriched sequentially for about 6 hours to enhance the nutritional quality of zooplankton while preventing significant degradation or nutrient depletion (Yuslan et al., 2021). After 6 hours of enrichment every day, the *M. micrura* was harvested and dried.

All prepared samples were pulverised using a mortar and pestle into a powder form (< 0.1 mm) (Suhaimi et al., 2022b). The analysis of crude protein and crude lipid was performed. The protein analysis involved placing approximately 0.2g of samples into glass tubes for the purpose of digestion. Three different methods—digestion, distillation, and titration—were used to determine the protein content. The percentage of protein was calculated by multiplying the N2 (%) percentage by an empirical value of 6.25. The total lipid content of the diets was assessed utilising the modified Bligh and Dyer (1959) method. Approximately 2g of each sample was weighed and underwent lipid extraction. The extraction solvent was evaporated, and the residual lipid fraction was collected in a pre-weighed cup. The cup, containing the extracted lipids, was desiccated to eliminate any remaining solvent prior to reweighing. The total lipid content was determined by the weight difference before and after extraction.

Growth, Survival and Reproduction of *Moina micrura*

The population growth of *M. micrura* was determined by counting the population of *M. micrura* once every two days for 15 days, which was equivalent to the average lifespan (cycle) of *M. micrura.* Aeration was turned off for 5-10 minutes prior to sampling to enable uniform distribution of the *M. micrura* within the culture medium. Sampling was conducted at several points within the culture container to address spatial variability, with each sample comprising a predetermined volume of 10mL collected from a minimum of four points. The *M. micrura* samples were observed in a counting chamber under the microscope.

The specific population growth rate (r) and survival rate were calculated by using Equation 1 and Equation 2 (Lee et al., 2013; Yuslan et al., 2021):

$$\text{Specific population growth rate (r)} = \left(\frac{\ln Ne - \ln Ni}{t}\right) \quad [1]$$

$$\text{Survival rate} = \left[\frac{\text{Total number of the sample taken}}{\text{Total number of initial } \textit{Moina} \text{ sp.density}}\right] \times 100\% \quad [2]$$

where Ni: initial density of *M. micrura*, Ne: end density of *M. micrura,* and t: cultured days.

The reproduction of *M. micrura* was determined with 5 individuals, and the experiment was conducted using the equation by Khoo et al. (2013) to measure reproduction, and the life tables were used to record reproduction data, which included gross reproduction rate, net reproduction rate, and generation time. These data were estimated as follows in Equations 1 to 5:

$$\text{Gross reproduction rate} = \sum m_x \quad [3]$$

$$\text{Net reproduction rate } (R_0) = \sum l_x \, m_x \quad [4]$$

$$\text{Generation time } (T) = \sum \frac{l_x \, m_x x}{R_0} \quad [5]$$

where, m_x: age-specific fecundity (number of neonates produced per surviving female at age x), and l_x: Proportion individual surviving to age x.

Growth Performance and Survival of *Macrobrachium rosenbergii* Post-larvae

At the start of the experiment, the initial body weight (BW) and initial body length (BL) of the post-larvae were measured and recorded; the same were done after 30 days of the experiment, where the final weight and final length of the post-larvae in each tank were measured and recorded in order to determine the survival rate and specific growth rate (SGR) of *M. rosenbergii* post-larvae of each treatment. After the 30-day experimental period, the survival rate and specific growth rate (SGR) of *M. rosenbergii* were individually determined using the subsequent Equations 6 to 7 (Asaduzzaman et al., 2016; Sumon et al., 2018):

$$\% \text{ Survival rate} = \frac{\text{No.of live PL}}{\text{No.of PL introduces}} \times 100 \quad [6]$$

$$\text{Specific growth rate (SGR)} = (\ln W_t - \text{In } W_i \times 100) / t \quad [7]$$

where W_i: initial weight of *M. rosenbergii* post-larvae, W_t: final weight of *M. rosenbergii* post-larvae, and t: cultured days.

Statistical Analysis

Before conducting the statistical analysis, all recorded data were subjected to a normality test. The Statistical Package for the Social Sciences (SPSS) software was utilised to execute a one-way analysis of variance (ANOVA) on the experimental data, and significant differences were determined employing the Tukey test ($P < 0.05$). Presentation of all data was done in the form of mean ± standard deviation.

RESULTS

Protein and Lipid Content of Diets and Enriched *Moina micrura*

Based on our results in Figure 1, yeast recorded the highest protein (51.41 ± 2.52%) compared to soybean (39.47 ± 1.27%), mung bean (21.08 ± 0.68%), and sesame seed, which was recorded as the lowest (17.16 ± 0.75%). For lipid content, sesame seed recorded the highest (26.61 ± 0.56%) compared to soybean (9.77 ± 0.77%), mung bean (1.02 ± 0.61%), and the least lipid content was recorded in yeast (0.93 ± 0.58%), as stated in Figure 2.

The highest protein content was *M. micrura* enriched with soybean (73.92 ± 0.13%), compared to *M. micrura* enriched with yeast (72.11 ± 0.30%), sesame seed (71.48 ± 0.24%), and the lowest was recorded in mung bean (69.02 ± 0.28%), as stated in Figure 3. The highest lipid content was recorded in *M. micrura* enriched with soybean (14.68 ± 0.65%), followed by *M. micrura* enriched with sesame seed (14.18 ± 0.31%), *M. micrura* enriched with yeast (12.81 ± 0.74%), and the lowest was recorded in *M. micrura* enriched with sesame seed, as stated in Figure 4.

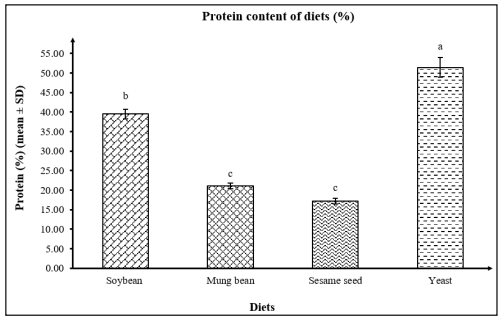


Figure 1. Protein content of diets. The small letters indicate significant differences between enrichments (P<0.05)

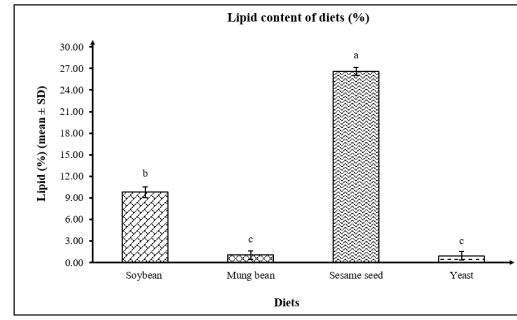


Figure 2. Lipid content of diets. The small letters indicate significant differences between enrichments (P<0.05)

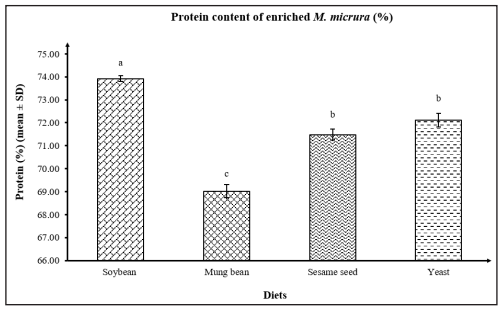


Figure 3. Protein content of enriched *M. micrura* fed with different diets. The small letters indicate significant differences between enrichments (P<0.05)

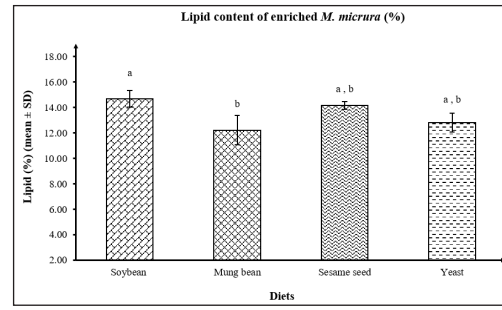


Figure 4. Lipid content of enriched *M. micrura* fed with different diets. The small letters indicate significant differences between enrichments (P<0.05)

Growth, Survival, and Reproduction of *M. micrura*

The results showed that *M. micrura* fed soybean had the highest population growth compared to the other diets, and the least suitable enrichment used was mung bean, as shown in Figure 5, since it had the lowest population growth. This study establishes that the population growth rate of *M. micrura* was maximised when enriched with soybean, whereas the lowest growth rate was observed for *M. micrura* enriched with mung bean.

The survival rate of *M. micrura* fed with soybean was (95 ± 4.9%), which is the highest compared to other enrichments, and sesame seed produced the lowest survival rate (78.38 ± 15.76%). For the specific population growth rate, soybean showed the highest result (0.059 ± 0.001%), followed by yeast (0.052 ± 0.002%), sesame seed (0.048 ± 0.002%), and mung bean (0.025 ± 0.004%). The results are shown in Table 1. The survival rate of each treatment is relatively similar, especially for sesame seed and yeast, while mung bean gave the least favourable result, and the results show a significant difference between each treatment ($P < 0.05$).

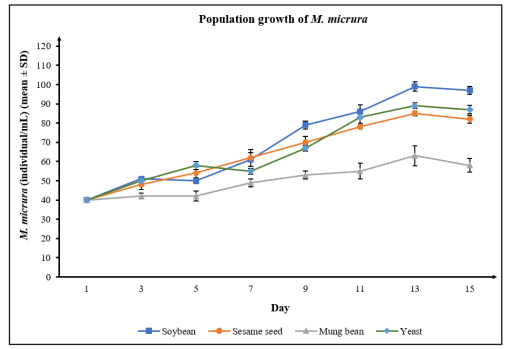


Figure 5. The population growth of *M. micrura* fed with different diets

Table 1
Survival rate and specific population growth rate of M. micrura *fed different enrichments*

Diets	Survival Rate (%)	Specific Population Growth Rate
Mung bean	78.38 ± 15.76[a]	0.025 ± 0.004[c]
Soybean	95.00 ± 4.90[b]	0.059 ± 0.001[a]
Sesame seed	86.88 ± 10.40[a, b]	0.048 ± 0.002[b]
Yeast	90.75 ± 9.05[a, b]	0.052 ± 0.002[b]

Note. All values are mean ± standard deviation (n=3). *Small letters indicate significant difference between treatments

The net reproduction rate (Ro) of *M. micrura* fed with soybean was (9.87 ± 3.78%), and it was comparatively equal to that of yeast (9.20 ± 2.8%). The same can be said of the gross reproduction rate, where soybean (2.67 ± 0.42%) has the best result, followed by yeast (2.47 ± 0.42%). Generation time for both soybean (4.78 ± 0.43 days) and yeast (4.68 ± 0.49 days) have insignificantly difference compared to mung bean (9.27 ± 0.45 days), as highlighted in Table 2. Mung bean has the lowest reproduction results.

Table 2
Net reproduction rate, gross reproduction rate and generation time of M. micrura *fed with different types of enrichments*

Diets	Net Reproduction Rate (%)	Gross Reproduction Rate (%)	Generation Time (Days)
Mung bean	2.20 ± 0.92[b]	0.73 ± 0.31[b]	9.27 ± 0.45[a]
Soybean	9.87 ± 3.78[a]	2.67 ± 0.42[a]	4.78 ± 0.43[b]
Sesame seed	6.67 ± 1.67[a, b]	2.00 ± 0.4[a]	5.80 ± 0.75[b]
Yeast	9.20 ± 2.80[a]	2.47 ± 0.42[a]	4.68 ± 0.49[b]

Note. All values are mean ± standard deviation (n=3). *Small letters indicate significant difference between treatments.

Protein and Lipid Content of *Macrobrachium rosenbergii* Post-larvae

Based on the results from Figures 6 and 7, the lipid and protein content in the *M. rosenbergii* body composition was highest in *M. rosenbergii* fed with soybean-enriched *M. micrura,* with the lipid content of 7.52 ± 0.55%, and protein content of 69.20 ± 0.77%. Meanwhile, *M. rosenbergii* fed with mung bean-enriched *M. micrura* had the lowest lipid content (4.38 ± 0.71%), and protein content (63.49 ± 0.44%).

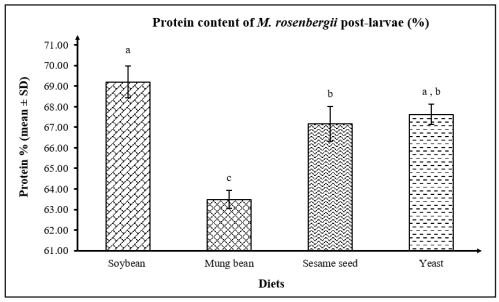


Figure 6. Protein content of *M. rosenbergii* fed with different legume-enriched *M. micrura.* The small letters indicate significant differences between enrichments (P<0.05)

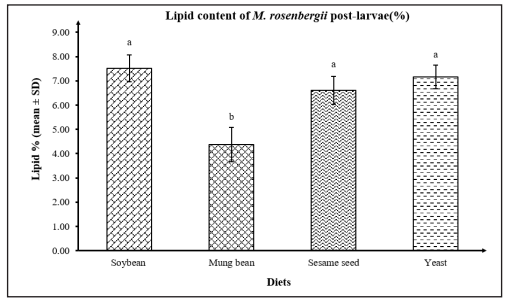


Figure 7. Lipid content of *M. rosenbergii* fed with different legume-enriched *M. micrura.* The small letters indicate significant differences between enrichments (P<0.05)

Growth performance and survival of *Macrobrachium rosenbergii* post-larvae

After 30 days of culture, specific growth rate and survival rate of *M. rosenbergii* post-larvae were calculated and presented in Table 3. The highest specific growth rate was obtained when the post-larvae were fed with soybean-enriched *M. micrura* (5.73 ± 0.21%) compared to other diets, while the lowest was obtained when fed with mung bean-enriched *M. micrura.* The highest survival rate was obtained in soybean-enriched *M. micrura* (87.67 ± 2.52%), followed by yeast-enriched *M. micrura* (86.33 ± 2.31%), sesame seed-enriched *M. micrura* (84.33 ± 1.53%), and mung bean-enriched *M. micrura* (78.33 ± 1.53%).

From the results obtained during the 30-day culturing period, the survival rate and specific growth rate of *M. rosenbergii*, when fed with four types of enriched Moina, exhibited discernible differences. *M. rosenbergii* fed with soybean-enriched *M. micrura* demonstrated the highest values for both survival rate (93.67 ± 1.53%) and specific growth rate (5.73 ± 0.21%), suggesting positive effects on the growth and survival of *M. rosenbergii* due to the soybean enrichment. This finding aligns with a study by Suhaimi et al. (2022a), which reported excellent growth performance in tilapia fry fed with *Moina macrocopa* enriched with rice bran and soybean, particularly in terms of weight gain.

Table 3
Specific population growth rate and survival rate of M. rosenbergii *fed with different enriched* M. micrura

Diets	Specific Growth Rate	Survival Rate (%)
Mung bean	3.84 ± 0.23^{c}	78.33 ± 1.53^{b}
Soybean	5.73 ± 0.21^{a}	87.67 ± 2.52^{a}
Sesame seed	4.85 ± 0.12^{b}	84.33 ± 1.53^{a}
Yeast	$5.26 \pm 0.37^{a, b}$	86.33 ± 2.31^{a}

Note. All values are in mean ± standard deviation (n=3). *Small letters indicate significant differences between treatments

DISCUSSION

The composition of protein and lipid in the feed utilised in this study unequivocally influences the corresponding protein and lipid content within Moina. Soybean, closely followed by yeast, exhibits heightened levels of protein and lipid, thereby augmenting the nutritional profile of *M. micrura* and concurrently contributing to the enhancement of their growth and reproductive development. The absorption of protein in Moina is high in soybean feed, with 73.92 ± 0.13% of protein level in enriched Moina, despite its lower protein content of 39.47 ± 1.27% compared to yeast with 51.41 ± 2.52%.

Research suggests that the complex composition of yeast cell walls, abundant in β-glucans and mannoproteins, could inhibit protein bioavailability. A study indicates that these components may decrease nutrient absorption in animals. In contrast, soybean meal possesses a more digestible protein composition and a well-balanced amino acid profile, resulting in improved protein absorption. This indicates that, despite yeast's high protein content, its structural complexity may restrict its efficacy as a protein source in comparison to soybean meal (Morales-López et al., 2009). Nevertheless, it is imperative to conclude that the feed employed for enriching *M. micrura* must align with the zooplankton's consumable suitability.

The absorption and fat content in *M. micrura,* as in Figure 4, do not significantly vary among cultures with feeds of differing fat content. The lipid content in Moina exhibits minimal variation when cultured with feeds of varying lipid content. This phenomenon can be related to Moina's selective absorption and metabolic regulation mechanisms, which sustain a relatively stable lipid composition despite fluctuations in dietary lipids.

A study by Shidik et al. (2021) found that although the overall lipid content in *Moina* sp. was largely stable across various feed treatments, the composition of specific fatty acids fluctuated based on the diet. For example, Moina cultivated with biofloc-based feeds demonstrated elevated levels of saturated fatty acids, while those nourished with Chlorella-based diets showed enhanced polyunsaturated fatty acids. This indicates that although Moina can adjust its fatty acid composition according to available dietary sources, its total lipid content remains consistent, underscoring the species' capacity to effectively regulate lipid absorption and metabolism.

This discrepancy suggests potential limitations in meeting the dietary requirements of *M. micrura* or its suitability as a feed source. Furthermore, there appears to be a dearth of research on zooplankton enrichment with mung bean in comparison to soybean and yeast, emphasising the need for comprehensive investigations. An additional insight emerges from a study by Fouzi et al. (2021) indicating that the combination of fishmeal and dry yeast exhibited the highest density across all sample days, underscoring the importance of specific enrichment combinations. To optimise the nutritional content in the bodies of *M. macrocopa*, it becomes imperative to augment their diet with feeds of high nutritional value, as emphasised by Suhaimi et al. (2022b).

Zooplanktons fed with modified diets have been proven to have excellent biochemical properties and enhance larval appetites and feeding (Olivotto et al., 2011). Similar observations were reported by El-Khodary et al. (2020), who identified that a diet primarily composed of soybeans led to the highest population density in *Cyclops abyssorum divergens*. Hakima et al. (2013) further supported these findings, highlighting that manipulating the quality and type of feed can significantly enhance the population growth of *Moina* sp., with soybean emerging as the most conducive for cultural development. The implications of this research extend to the broader context of aquaculture hatcheries, emphasising that the sustainability and successful production of live feed hinge upon the specific type of feeding utilised.

This study observed that the growth of *M. micrura* decreased significantly in mung bean-fed enrichment, presumably due to the inferior nutritional value of mung beans relative to other enrichments. Furthermore, mung beans are rich in carbohydrates but may be deficient in essential fatty acids and proteins required for optimal *Moina* reproduction and growth. Mung beans also possess anti-nutritional compounds, including tannins, phytic acid, and trypsin inhibitors, which may hinder digestion and nutrient absorption (Cheng & Langrish, 2025). Higher specific population growth rate (r-values) might result from reduced generation times or enhanced fecundity (Khoo et al., 2013).

Notably, enriched *M. micrura* with the highest protein and lipid content also demonstrated the highest observed survival rate among the various feeds examined in the study. According to Azani et al. (2022), enhancing zooplankton can be achieved by introducing different types and levels of nutrients into their diets, a notion supported by both Sarma et al. (2020) and Damayanti et al. (2020).

Concerning the reproductive aspects of *Moina* sp., it is evident that soybean-enriched *M. micrura* exhibits superior outcomes for net reproduction rate, generation time, and gross reproduction rate. This underscores the efficacy of soybean as the most suitable enrichment for enhancing the reproduction of *M. micrura.* In alignment with the research conducted by Azuraidi et al. (2013), tropical Cladocera, including

Moina sp., demonstrate a rapid maturation process, reaching maturity as early as day 3, consequently leading to heightened reproductive rates. The reproductive success of Cladoceran offspring is notably impacted by the introduction of various enrichment types, as highlighted by Azani et al. (2022).

Crucial nutrients such as protein and lipids play pivotal roles in the reproductive processes of *M. micrura.* In accordance with Fink et al. (2011), elevated levels of the diet's nutrient content significantly influence reproduction process in cladocera. Proteins are essential for egg development and overall reproductive success, while lipids serve as an energy source and are vital for the formation of reproductive tissues. Feeds with suitable protein and lipid contents contribute to a high reproduction rate, with soybeans proven to be the most effective enrichment for manipulating the reproduction rate of *M. micrura* compared to other enrichments utilised in this study. The different ways soybean and mung bean enrichment affect reproduction highlight the importance of recognising the specific nutrients in various legumes. This understanding is crucial in meticulously developing feeds that provide the best conditions for zooplankton culture in aquaculture.

Upon subjecting *M. rosenbergii* to varying enriched *M. microra* diets, discernible differences in the protein and lipid composition of *M. rosenbergii* were observed but the protein and lipid profiles of the post-larvae are comparable as the protein and lipid profiles of Moina. When it consumes Moina as feed, their protein and lipid profiles may closely mirror those of Moina due to nutrient assimilation. Research indicated that the dietary habits of aquatic organisms substantially influence their biochemical composition.

Protein and lipid sources in a prawn's diet can influence their body composition, encompassing cholesterol levels and fatty acid profiles. Research on juvenile Pacific white prawns (*Litopenaeus vannamei)* indicated that substituting fish meal and fish oil with soybean meal and soy oil did not impact growth performance but decreased body cholesterol levels (Cheng & Hardy, 2004). Notably, those fed with soybean-enriched *M. micrura* exhibited the highest values for both protein and lipid content. Protein and lipid are pivotal nutrients known for their substantial roles in larval development (Radhakrishnan et al., 2020). As articulated by Bhavan et al. (2010), protein is imperative for the formation, maintenance, and normal function of bodily tissues in *M. rosenbergii*. Furthermore, lipids serve as the primary energy source through metabolic processes.

Although the utilisation of zooplankton, particularly *Artemia*, is prevalent in larval production, alternative zooplankton such as *M. micrura* and copepods can serve as viable substitutes, exerting an influence on the nutritional composition of the cultured species (Joshua et al., 2022). Previous studies have reported an elevation in protein, carbohydrate, and lipid levels in *Catla catla* fry when exposed to various live feeds, including cyclopoid *Thermocyclops decipiens* and cladoceran *M. micrura* (Manickam et al., 2020). The use of enriched *M. micrura* as a live fish food has been demonstrated to enhance the nutritional

content of *M. rosenbergii*, affirming its potential as a valuable dietary component in aquaculture practices.

A study by Huang et al. (2017) demonstrated analogous patterns in growth and physiological development when dietary fishmeal was substituted with soybean meal in their diet composition. While the soybean treatment yielded the highest growth performance and survival rate for *M. rosenbergii*, it is noteworthy that the yeast and sesame seed treatments also produced favourable outcomes in terms of both survival rates and specific growth rates. The growth rate assumes a pivotal role in shaping the survival outcomes of *M. rosenbergii*. A superior growth rate is typically indicative of heightened overall robustness and resilience within *M. rosenbergii*, potentially resulting in elevated survival rates.

In a study conducted by Sukri et al. (2016), heightened nutritional formulations were observed to correlate with increased survival rates and enhanced growth of *M. rosenbergii*. Notably, *M. rosenbergii* subjected to a diet enriched with soybean-fed *M. micrura* exhibited the highest survival rate, underscoring the efficacy of utilising highly nutritious feeds to augment the survival of the cultured species. The disparities in growth and survival rates of *M. rosenbergii* post-larvae can be attributed to divergent preferences in nutritional quality, feeding habits, and digestive dynamics.

The early post-larval phases of *M. rosenbergii* are notably influenced by the types of feeds provided, and the nutritional enrichment of *M. micrura* prior to fish larvae feeding can be effectively achieved through diverse enrichment strategies (Aziz et al., 2023). Given that the post-larval phases of *M. rosenbergii* are predominantly predatory and reliant on live food sources (Das et al., 2007; Martin et al., 2006; Sornsupharp et al., 2013), the observed variations in growth and survival underscore the critical importance of tailored nutritional strategies during these developmental stages.

CONCLUSION

This study concluded that the enrichment of *M. micrura* with soybean significantly improved its nutritional profile, resulting in enhanced growth and survival rates of *M. rosenbergii* post-larvae. The enrichment positively affected the development and reproduction of *M. micrura,* thereby confirming the effective transfer of nutrients within the food chain. The outcomes achieved the research objectives by confirming the advantages of nutritional enhancement in live feed. The results indicate the need for additional investigation into plant-based enrichments and provide practical insights for enhancing larviculture protocols in aquaculture.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENTS

AHS conducted the research and prepared the manuscript. AA assisted with the research and manuscript preparation. HJL, NAH, and SRMY contributed to the study design and manuscript revision. TPA and ASM provided technical input and contributed to the critical review of the manuscript. NWR conceived and supervised the study and critically revised the manuscript. All authors read and approved the final manuscript.

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Ameliorating Acidity and Nutrient Leaching in Tropical Soils Using Silico-Manganese Slag in Combination with Chemical Fertilisers

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ABSTRACT

High rainfalls and humid temperatures in tropical regions resulted in acidic soils because of excessive leaching of base cations and occupation of iron (Fe^{2+}) and aluminium (Al^{3+}) ions. Silico-manganese slag (SiMn slag) is an alkaline material which could neutralise soil acidity and regulate nutrient leaching. A 30-day leaching study was conducted to determine the effects of SiMn slag and chemical fertilisers (CF) on nutrient leaching in Bekenu series (*Typic Paleudults*). One kilogramme of Bekenu series was mixed with different rates of SiMn slag and CF: T0 = soil only; T1 = SiMn slag only; T2 = soil + 100% CF; T3 = soil + 100% SiMn slag; T4 = soil + 75% SiMn slag + 25% CF; T5 = soil + 50% SiMn slag + 50% CF; and T6 = soil + 25% SiMn slag + 75% CF. The co-application of SiMn slag and CF consistently increased pH and EC of the leachates, which resulted in significantly higher amounts of ammonium (NH_4^+), nitrate (NO_3^-), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) leached. After 30 days of leaching, co-applying SiMn slag and CF increased soil pH, electrical conductivity (EC), exchangeable NH_4^+, Ca^{2+}, and Mg^{2+} by reducing exchangeable acidity and Al^{3+}. We concluded that SiMn slag did not mitigate nutrient leaching, but rather it may have ameliorated acidity to improve nutrient availability. The most suitable combinations are T5 (soil + 50% SiMn slag + 50% CF) and T6 (soil + 25% SiMn slag + 75% CF). Further pot and field studies are recommended to evaluate soil–plant interactions using SiMn slag.

Keywords: Highly weathered, nutrient retention, scheduled waste, silicon, soil fertility and management, Ultisols, *Zea mays* L

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INTRODUCTION

Excessive nutrient leaching from the soil poses a significant impediment to optimal plant growth and nutrient utilisation, in addition to affecting agricultural productivity, because the plant-available nutrients percolate to the underground below the root zone when prolonged rainfall exceeded field capacity (Lehmann & Schroth, 2003). It is reported that approximately 3.95 billion hectares of land are acidic soils in the world (Easton & Bock, 2016; Rashmi et al., 2017). In the tropical regions, excessive rainfalls and humid temperatures resulted in the formation of highly weathered acidic soils such as Ultisols and Oxisols (Paramisparam et al., 2021). Narro et al. (2001) also pointed out that the global distribution of the Oxisols and Ultisols is in the following order: America (40.9%), Asia (26.4%), Africa (16.7%), Europe (9.9%), and Australia and New Zealand (6.1%). These acidic soils are characterised by low pH levels with an abundance of kaolinite clay minerals, aluminium and iron oxides; low in pH buffering capacity, base saturation, and nutrient availability, as well as prone to nutrient leaching (Kuo et al., 2020; Shamshuddin & Wan, 2011). In Malaysia, approximately 72% of agricultural lands are classified as highly weathered acidic soils, which impair crop productivity because of their low pH (<5.5) and relatively poor fertility due to nutrient imbalance (Jusop & Ishak, 2010).

A promising avenue to address the issue of acidic soils is the exploration of innovative soil amendments that not only mitigate acidity, Al and Fe toxicities, but also improve nutrient retention in acidic soils. Recently, many promising and novel strategies for enhancing the productivity in Malaysian soils, aimed at increasing crop yields and economic income for both small and large-scale farmers, involves soil conditioning through the incorporation of organic and inorganic amendments, have been explored (Hassan et al., 2023; Lee et al., 2021; Ali et al., 2023; Omar et al., 2021; Ch'ng et al., 2019). However, the potential of scheduled waste such as silico-manganese slag (SiMn slag) as a soil amendment to augment the physical, chemical, and biological properties of acidic soils in Malaysia remains unrevealed. Silico-manganese slag is a by-product generated during the production of SiMn alloys, which is primarily used in steel manufacturing, where approximately 15% to 20% of total steel production results in the formation of SiMn slag (Ting et al., 2023; Wu et al., 2007). Notably, the town of Bintulu in Sarawak, Malaysia, is known for its industrial activities, including SiMn production with an annual tonnage of approximately 444,000 tonnes in 2022 (Ting et al., 2023). Despite its industrial significance in construction and building materials, the full potential of SiMn slag, its properties, and its various applications in agriculture remain largely unexplored. In this context, conducting localised research can unveil valuable insights into the characteristics of this slag, its environmental implications, and innovative approaches to manage it effectively, thereby fostering sustainable industrial development in the region.

The reason why SiMn slag is being distinguished as an effective soil conditioner is because of its advantageous qualities, such as neutralising capabilities due to its alkalinity, ease of application, and considerable amounts of alkaline materials such as silicon oxide (26.19% to 47.54%) (Mussawar et al., 2022; Wang et al., 2017b), calcium oxide (9.24% to 29.10%) (Navarro et al., 2020; Wang et al., 2017b), and magnesium oxide (3.70% to 6.91%) (Cheng et al., 2023; Criado et al., 2018) contents. Beyond its liming effects to improve soil pH, SiMn slag is reputed to detoxify soil from excessive Al^{3+} and Fe^{2+} saturations, which can be detrimental to plant roots and inhibit phosphorus (P) uptake by plants (Boguta et al., 2019; Jo et al., 2022). Many research has also reported that the increase of water-soluble Si content in acidic soils not only mobilises P via anion exchange reaction, but it also mitigates P fixation by transforming active Al^{3+} and Fe^{2+} into insoluble hydroxides via the increased pH (Ch'ng et al., 2014; Schaller et al., 2019). Cai et al. (2015) also opined that the inclusion of alkaline materials, which have higher base saturation compared with the unproductive acidic soils, can improve the availability of potassium (K^{+}), calcium (Ca^{2+}), and magnesium (Mg^{2+}) in addition to immobilising excessive H^{+} in the soil solution. Furthermore, SiMn slag contains silicon (Si), a beneficial nutrient known to enhance plant growth and yield by improving resistance to environmental stressors, nutrient imbalances, pathogens, and herbivores (Neu et al., 2017; Sahebi et al., 2015; Wang et al., 2017a). The application of Si-based soil amendments significantly improved growth, development, and reproduction of rice and maize, which are being categorised as Si accumulators (Pozza et al., 2015; Sahebi et al., 2015; Shajarutulwardah et al., 2023). Moreover, applying SiMn slag to mineral soils can release Si to enhance soil properties, including strength, structure, water movement, air circulation, and nutrient retention and availability (Schaller et al., 2019; Schaller et al., 2020).

Based on the aforementioned rationales, there is a dearth of information on the effects of combined use of SiMn slag and chemical fertilisers to mitigate nutrient leaching and improve nutrient retention in acidic soils such as the Bekenu series. Moreover, the nutrient retention and leaching in the acidic soil following the application of SiMn slag are sparsely explored. Thus, it was hypothesised that the use of SiMn slag can neutralise soil acidity, detoxify active Al^{3+} and Fe^{2+} in addition to improving nutrient retention in acidic soils via increased contents of CaO, MgO, and SiO_3^{2-}. The objective of this study was to determine the effect of SiMn slag application combined with chemical fertilisers on nutrient leaching in Bekenu series (*Typic Paleudults*). It is hoped that the inclusion of SiMn slag can reduce the reliance of farmers on the heavy use of chemical fertilisers in sustaining a productive maize farming system via a soil health rejuvenation approach.

MATERIALS AND METHODS

Soil Sampling and Preparation

Based on the United States Department of Agriculture (USDA) soil classification system, the soil used in this leaching experiment was the Bekenu series (*Typic Paleudults*). This soil was selected because it is acidic and commonly used for oil palm, rubber, and black pepper cultivations (Paramananthan, 2020). According to Paramananthan (2020), the Bekenu series is an Ultisol which has fine loamy soil texture with more than 90% silica minerals, and it has a well-drained soil. In terms of parent material, the soil is made up of red-yellow sedimentary rocks with more than 30% ferric oxide, which contributes to its highly acidic nature. Moreover, Shamshuddin & Wan (2011) opined that high rainfall and humid temperatures result in the formation of highly weathered soils prone to leaching of base cations. In terms of microclimate, the soil sampling area has an average annual rainfall, air temperature, and relative humidity of 2,993 mm, 27 °C, and 80%, respectively (Johan et al., 2021). The soil sample was collected using a shovel at an uncultivated area of Universiti Putra Malaysia Sarawak (UPMS) (latitude 03°20 N, longitude 113°07 E). The soil sample was collected in a pedon (1 m length × 1 m width) at 20 cm depth, followed by air-drying, manual crushing, and passing a 2.0 mm sieve prior to the determination of selected physico-chemical properties.

Silico-Manganese Slag Preparation

The silico-manganese slag (SiMn slag) is a scheduled waste from steel production at Pertama Ferroalloys Sdn. Bhd., Bintulu, Sarawak, Malaysia (latitude 03°54 N, longitude 113°31 E). It was selected for amending the nutrient leaching in the Bekenu series because of its alkalinity to neutralise soil acidity and increase the number of exchangeable sites to retain the nutrients. The SiMn slag was randomly collected into gunny sacks using a shovel and then transported to the Soil Science Laboratory under the Department of Crop Science in UPMS for air-drying, manual crushing, and passing through a 2.0 mm sieve before the physico-chemical properties characterisation.

Initial Characterisation of Soil and Silico-Manganese Slag

Before commencing the soil leaching experiment, the physico-chemical properties of the soil and SiMn slag were determined for initial characterisation. Details of the analysis methods for determining the physico-chemical properties of the soil and SiMn slag are summarised in Table 1. The initial physico-chemical properties of the soil and SiMn slag were summarised in Table 2. The prepared sample of SiMn slag was sent to an external laboratory (Chemsain Konsultan Sdn. Bhd., Kuching, Sarawak, Malaysia) for the determination of heavy metals such as lead (Pb), nickel (Ni), cadmium (Cd), chromium (Cr), mercury (Hg), and arsenic (As). The composition and regulatory threshold of each heavy metal are reported in Table 3.

Table 1
Methods used in analysing the physico-chemical properties of soil and silico-manganese slag

No.	Physico-Chemical Analyses	Method	References
1	Soil bulk density	Core ring method	Tan, 2005
2	Soil texture and particle size distribution	Hydrometer method	Bouyoucos, 1962
3	pH in water and potassium chloride (KCl)	Potentiometric method	Peech, 1965
4	Electrical conductivity	Potentiometric method	Peech, 1965
5	Total organic matter	Loss-on-ignition method	Chefetz et al., 1996
6	Total organic carbon	Loss-on-ignition method	Chefetz et al., 1996
7	Cation exchange capacity	Leaching method using 1.0 mol dm^{-3} ammonium acetate at pH 7, followed by steam distillation	Bremner & Keeney, 1965; Cottenie, 1980
8	Total nitrogen	Kjeldhal method	Bremner, 1965; Keeney & Nelson, 1982
9	Exchangeable ammonium and available nitrate	Extraction using 2 mol dm^{-3} KCl, followed by steam distillation	Keeney & Nelson, 1982
10	Total phosphorus and cations	Aqua regia method	Bernas, 1968
11	Available phosphorus	Mehlich's double acid method, followed by the blue colour method	Mehlich, 1953; Murphy & Riley, 1962
12	Exchangeable cations	Mehlich's double acid method, followed by Atomic Absorption Spectroscopy determination	Mehlich, 1953
13	Total titratable acidity, exchangeable aluminium, and hydrogen	Titration method	Rowell, 1994
14	Silicon oxide	Inductively Coupled Plasma	-

Table 2
Initial physico-chemical properties of Bekenu series and silico-manganese slag

Physico-Chemical Properties	Value Obtained	
	Bekenu Series	Silico-Manganese Slag
Soil texture (USDA)	Sand: 48.3% Silt: 24.0% Clay: 27.7% Sandy clay loam	n.d.
Particle size distribution	n.d.	0.05-2.0 mm: 57.32% 0.002-0.05 mm: 4.58% <0.002 mm: 38.10%
Bulk density (g cm^{-3})	1.20	n.d.
pH in water	5.13	8.00
Electrical conductivity (µS cm^{-1})	90	3600

Table 2 (*continued*)

Physico-Chemical Properties	Value Obtained	
	Bekenu Series	Silico-Manganese Slag
	$cmol_{(+)}kg^{-1}$	
Cation exchange capacity	7.70	12.00
Exchangeable acidity	1.21	0.10
Exchangeable Al^{3+}	1.03	0.06
	$mg\ kg^{-1}$	
Exchangeable NH_4^+	28.02	0.70
Available NO_3^-	15.88	1.00
Available P	1.24	0.90
Exchangeable K^+	76.0	240.8
Exchangeable Ca^{2+}	17.1	1391.6
Exchangeable Mg^{2+}	20.4	495.6
	%	
Silicon oxide (%)	n.d.	27.3
Organic matter content	7.24	4.00
Organic carbon content	4.20	2.32

Note. n.d. = not determined

Table 3
Heavy metal composition and regulatory threshold of silico-manganese slag

Heavy Metals	Value Obtained ($mg\ kg^{-1}$)	*Regulatory Threshold ($mg\ kg^{-1}$)
Lead (as Pb)	2.3	36.0
Nickel (as Ni)	8.9	28.9
Cadmium (as Cd)	Not detected (<1.5)	0.09
Chromium (as Cr)	4.2	14.4
Mercury (as Hg)	Not detected (<0.1)	0.42
Arsenic (as As)	4.6	43.0

Note. *The regulatory threshold is referred to the data from the Department of Environment Malaysia (2009)

Soil Leaching Experiment

From the bulked soil, one kilogramme (1 kg) of soil was weighed using an electronic balance in a cylindrical plastic container (15 cm diameter × 12 cm height) perforated with nine holes at the bottom of the container for each replicate. A piece of Whatman Grade 2 filter paper was added at the bottom of the container to prevent the leaching of soil particles during the commencement of the leaching experiment. After the soil physico-chemical characterisation, different rates of SiMn slag and chemical fertilisers (CF) were fixed in accordance with Malaysian Agricultural Research and Development

Institute (MARDI) recommendations for maize (*Zea mays* L.; Thai super sweet hybrid F1 variety) as our test crop for the future greenhouse and field trials. The recommended rates of nitrogen, phosphorus, and potassium sources supplied to the maize plants per hectare basis are 60 kg ha^{-1} N; 60 kg ha^{-1} P_2O_5; 40 kg ha^{-1} K_2O (130 kg ha^{-1} urea; 130 kg ha^{-1} Triple Super Phosphate; 67 kg ha^{-1} Muriate of Potash), respectively (MARDI, 1993). In this soil leaching experiment, based on the planting density of approximately 26,667 maize plants per hectare, the application rate of the mixture of straight fertilisers (urea, TSP, and MOP) was then scaled down to a per-plant basis at 12.26 g. Afterwards, varying rates by 25% (100%, 75%, 50%, 25%, and 0%) on the combined use of SiMn slag and chemical fertilisers were used to determine the effects of the treatments on regulating nutrient leaching in the soil and to reduce the reliance on chemical fertilisers in maize cultivation. Thus, the rates of the SiMn slag and chemical fertilisers were varied at 12.26 g (100%), 9.20 (75%), 6.13 g (50%), and 3.07 g (25%). Moreover, there were two additional treatments in the study to elucidate the treatment effects in this leaching study: soil only (T0) and SiMn slag only (T1). A total of seven treatments were used in this soil leaching experiment, and details of the treatments evaluated are summarised as follows:

- T0: 1 kg soil only
- T1: 1 kg silico-manganese slag only (SiMn slag)
- T2: 1 kg soil + 12.26 g chemical fertilisers (100%)
- T3: 1 kg soil + 12.26 g SiMn slag (100%)
- T4: 1 kg soil + 9.20 g SiMn slag (75%) + 3.07 g chemical fertilisers (25%)
- T5: 1 kg soil + 6.13 g SiMn slag (50%) + 6.13 g chemical fertilisers (50%)
- T6: 1 kg soil + 3.07 g SiMn slag (25%) + 9.20 g chemical fertilisers (75%)

The soil was mixed thoroughly with different rates of SiMn slag and chemical fertilisers, after which the mixture was moistened to 60% water holding capacity of the soil, which represents the field capacity in this leaching experiment. This is equivalent to 300 mL of distilled water, and the moistened soil samples were left overnight. For each experimental unit, a 500 mL beaker was placed under the perforated container for leachate collection at three-day intervals over the 30 days of leaching. The following day, at the start of the soil leaching experiment, another 300 mL of distilled water was poured meticulously onto the moistened soil sample and allowed to leach for three days into the 500 mL beaker for subsequent laboratory analysis. The addition of 300 mL of distilled water was repeated ten times at three-day intervals for leachate collection up to 30 days. Leachates were collected and analysed for selected chemical properties, which include pH, EC, and the leached amounts of nutrients (NH_4^+, NO_3^-, P, K^+, Ca^{2+}, and Mg^{2+}). After the leaching experiment, soil samples were air-dried, manually crushed, and passed through a 2 mm sieve for chemical analyses. The chemical analyses included soil pH in water, pH

in KCl, exchangeable acidity and Al^{3+}, electrical conductivity, cation exchange capacity, exchangeable NH_4^+, available NO_3^-, available P, and exchangeable Ca^{2+} and Mg^{2+} using the procedures as described in Table 1.

Experimental Design and Statistical Analysis

There were seven treatments evaluated in this soil leaching experiment, which were arranged in a Completely Randomised Design (CRD) with three replicates for each treatment. The data were analysed using one-way Analysis of Variance (ANOVA) to determine the treatment effects, followed by mean comparisons using Tukey's HSD test at $p \leq 0.05$ using Statistical Analysis System (SAS) version 9.4.

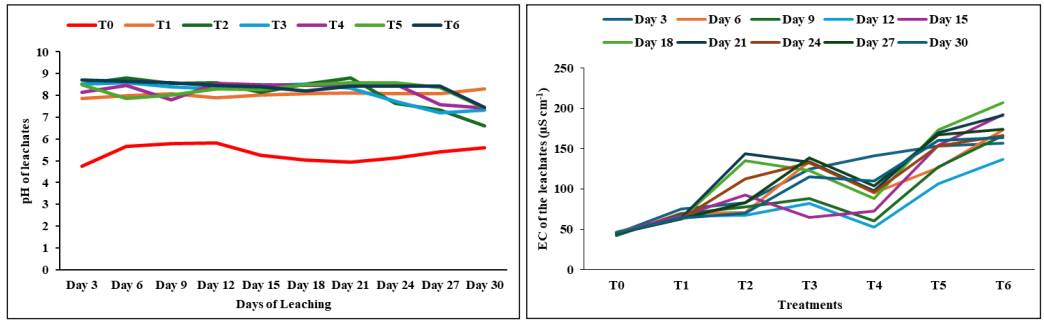
RESULTS AND DISCUSSION

Co-Application of Silico-Manganese Slag and Chemical Fertilisers on pH and Electrical Conductivity of Leachates

The pH of leachates for the SiMn slag only (T1) and the soils treated with SiMn slag and chemical fertilisers (T2, T3, T4, T5, and T6) exceeded 7, whereas that of the soil only (T0) was acidic (Figure 1a). Although it was observed that the pH of leachates from the soil with sole application of chemical fertilisers (T2) was above 7 in the initial days of leaching, which could be due to urea hydrolysis (Hamidi et al., 2021), the pH of leachate in T2 gradually decreased to 6.61 at day 30 of leaching. The findings suggest that the alkalinity of SiMn slag not only neutralises acidity and increases the pH of soil solution, but it also maintains the soil pH over 30 days of leaching (Ng et al., 2024b; Ng et al., 2022). Moreover, the dissolution of SiMn slag releases K^+, Ca^{2+}, Mg^{2+}, Na^+, and SiO_4^{4-} to increase the pH of leachates (Antonangelo et al., 2017; Opala et al., 2012; Ramos et al., 2006; Zhang et al., 2016), thus impeding Al^{3+}, Fe^{2+}, and Mn^{2+} hydrolysis reactions from producing H^+. Notably, the pH of leachates from soil only (T0) increased from day 6 to day 12, followed by a decrease in the pH of leachates on day 15 to day 21. In the soil solution, the dissolution of silicate minerals in the Bekenu series may have released SiO_4^-, which hydrolyses to form silicic acid (H_4SiO_4) and releases OH^- ions to neutralise free H^+ ions (Elisa et al., 2015). This could account for the pH increase of leachate in T0 from day 6 to day 12.

In Figure 1b, the soil only treatment (T0), consistently recorded the lowest EC throughout the experiment. The EC of leachates for T0 increased from 42.77 µS cm^{-1} on day 6 to 46.50 µS cm^{-1} on day 15 and decreased to 44.70 µS cm^{-1} on day 21, 44.50 µS cm^{-1} on day 24, and a slight increase on day 30 (45.47 µS cm^{-1}). The lowest EC in T0 was due to the low base cation saturations because ferric oxide is the parent material of the Bekenu series that is weathered into kaolinite clay minerals, which have low nutrient holding capacity (Paramisparam et al., 2021). On the contrary, the EC of leachates from SiMn slag (T1) were higher compared with soil only (T0) over 30 days of leaching, which

could be due to the dissolution of SiMn slag to release K^+, Ca^{2+}, Mg^{2+}, Na^+, and SiO_4^{4-} into the soil solution, suggesting that SiMn slag had higher EC compared with the Bekenu series. Regardless of the application rate of SiMn slag, the EC of leachates did not exceed the salinity threshold (4000 µS m^{-1}), indicating that the application rates of SiMn slag used in this leaching experiment will not cause salinity problems in the cropping system (USDA, 2011). Moreover, it was noted that the EC of leachates from the soils applied with chemical fertilisers (T2, T4, T5, and T6) were higher compared with T0 and T1. This finding implies that the combined use of chemical fertilisers such as urea, TSP, and MOP may significantly influence the EC of soil solution.



(a) (b)

Figure 1. (a) pH and (b) electrical conductivity of leachates in relation to treatments over thirty days of leaching
Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF

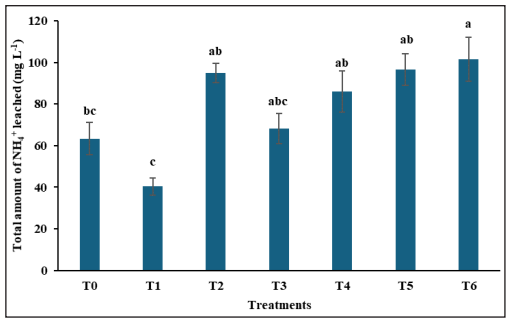
Co-Application of Silico-Manganese Slag and Chemical Fertilisers on Nutrients in the Leachates

The total amounts of NH_4^+ and NO_3^- leached were lower for the SiMg slag only (T1) compared with those of the soil with sole application of chemical fertilisers (T2) and the combined use of SiMn slag and chemical fertilisers (T4, T5, and T6) (Figures 2a and 2b). This is because the SiMn slag had lower exchangeable NH_4^+ (0.7 mg kg^{-1}) and available NO_3^- (1.0 mg kg^{-1}) compared with those of the Bekenu series at 28.02 mg kg^{-1} and 15.88 mg kg^{-1}, respectively (Table 2), suggesting that the combined use of nitrogen source fertilisers and SiMn slag is essential for optimum maize growth and productivity. It was observed that the total amount of NH_4^+ leached from the soils amended with 25% SiMn slag and 75% chemical fertilisers (T6) was higher compared with T0, whereas there is no significant difference between the cumulative amounts of NH_4^+ leached from the SiMn slag (T1) and the soil treated with 12.26 g of SiMn slag (T3), as presented in Figure 2a. This finding suggests that the NH_4^+ and NO_3^- availability in the soil solution was influenced by the chemical fertilisers rather than SiMn slag.

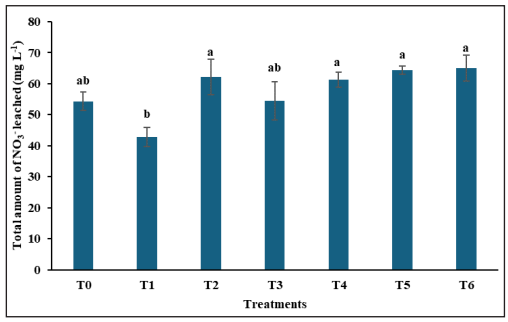
The significantly higher amounts of NH_4^+ and NO_3^- leached are indications of increased N availability in the soil solution, which could be due to the higher pH and EC of the leachates from the soils amended with SiMn slag and chemical fertilisers. The pH and EC are indications of the increased leached amounts of NH_4^+ and NO_3^- because this finding is in agreement with the findings of Neina (2019) and Zhang et al. (2018) who reported that the improved soil pH can mineralise organic N into inorganic NH_4^+ and NO_3^- in the soil solution. This suggests that SiMn slag can also increase N availability in acidic soils for optimum crop uptake through improved soil pH.

Unexpectedly, although it is reported that the increased pH improves microbial activities for mineralising P in the soils (Mehnaz et al., 2019; Malik et al., 2012; Opala et al., 2012), the inclusion of SiMn slag and chemical fertilisers did not significantly influence the total amounts of P leached over 30 days of leaching (Figure 2c). This is because SiMn slag had lower available P (0.90 mg kg^{-1}) compared with that of the soil (1.24 mg kg^{-1}) (Table 2). Moreover, the availability of P in soil solution could be affected by active Al^{3+}, Fe^{2+}, Ca^{2+}, and Mg^{2+}. In acidic soils (T0), active Al^{3+} and Fe^{2+} can adsorb with P (Boguta et al., 2019; Ifansyah, 2014; Zu et al., 2012), whereas the increased concentrations of Ca^{2+} and Mg^{2+} via the inclusion of SiMn slag can precipitate with plant-available P in the soil solution (Chien et al., 2012; Patle et al., 2019). These findings suggest that the effects of Al^{3+}, Fe^{2+}, Ca^{2+}, and Mg^{2+} might have diminished the effects of SiMn slag on improving P availability in the soil solution, regardless of treatment.

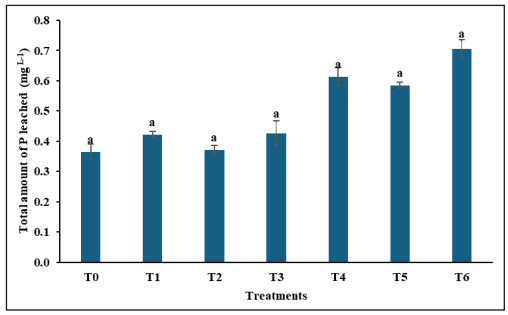
Generally, the total amounts of K^+, Ca^{2+}, and Mg^{2+} in the leachates over 30 days of leaching from the soils amended with SiMn slag and chemical fertilisers (particularly in T5 and T6) were higher compared with those of soil only (T0) as presented in Figures 3a, 3b, and 3c. The lowest total amounts of K^+, Ca^{2+}, and Mg^{2+} leached from T0 were due to the acidic nature of the Bekenu series. Compared with the soils with the sole application of chemical fertilisers (T2) (100% CF), the higher total amounts of K^+ leached from the soils treated with 50% SiMn slag and 50% CF (T5) and 25% SiMn slag and 75% CF (T6) suggest that the leaching of K^+ could be due to the antagonistic relationship between K^+ and Ca^{2+}. This finding is in agreement with that of Malvi (2011), who reported that the inclusion of amendments with high Ca^{2+} content can result in increased K^+ leaching due to the cation displacement process. Compared with T5 and T6, the significantly lower total amounts of K^+, Ca^{2+}, and Mg^{2+} leached from the soils treated with the sole application of SiMn slag (T3) also suggest that the dissolution of SiMn slag releases extremely low amounts of base cations over 30 days of leaching, although it was hypothesised that the SiMn slag can contribute to the increased amounts of base cations in the leachates.



(a)



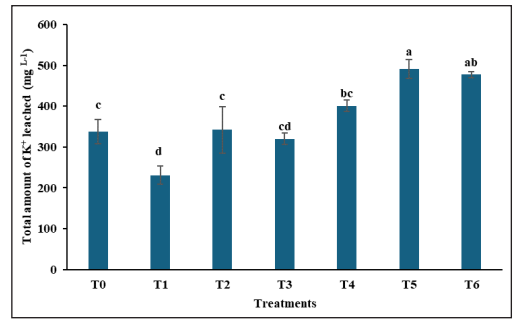
(b)



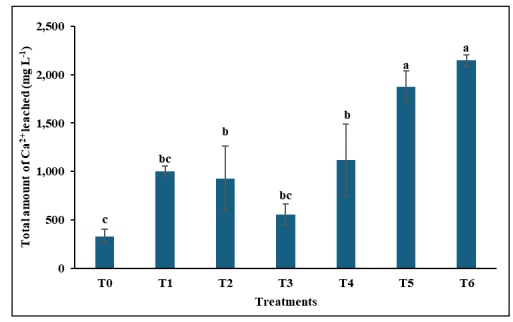
(c)

Figure 2. Total amount of (a) ammonium; (b) nitrate; and (c) phosphate ions in the leachates in relation to treatments after thirty days of leaching. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

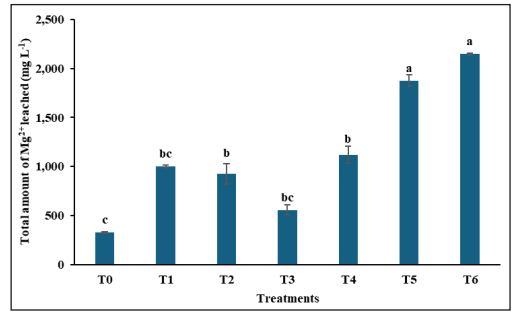
Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF



(a)



(b)



(c)

Figure 3. Total amount of (a) potassium; (b) calcium; and (c) magnesium ions in the leachates in relation to treatments after thirty days of leaching. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF

Co-Application of Silico-Manganese Slag and Chemical Fertilisers on Soil pH, Exchangeable Acidity, and Aluminium After Thirty Days of Leaching

The SiMn slag (T1) had the highest pH in water and KCl, followed by the soil pH in water and KCl of the soils amended with SiMn slag (T3, T4, T5, and T6), which were in the range of 6.31 to 6.59 and 4.74 to 5.15, respectively (Figures 4a and 4b). On the contrary, the pH in water and KCl of the soil only without any amendment (T0) were the lowest, whereas the pH and water and KCl of the soils amended with 100% chemical fertilisers (T2) were significantly lower compared with T3, T4, T5, and T6. This finding indicates that the alkalinity and application rates of the SiMn slag used in this study can ameliorate soil acidity and significantly improve soil pH.

The significant increment in soil pH following the application of SiMn slag could be related to the dissolution of calcium oxide (CaO), which can react with the H^+ in the soil solution to produce Ca^{2+} and water (Navarro et al., 2020; Navarro et al., 2017; Wang et al., 2017b). Moreover, the addition of amendments with higher saturations of base cations (K^+, Ca^{2+}, and Mg^{2+}) compared with the Bekenu series can immobilise active H^+ (Cai et al., 2015). When SiMn slag was incorporated into acidic soils, the dissolution of SiO_2 releases OH^- into the soil solution to elevate soil pH (Chong et al., 2022; Ning et al., 2016). This is because the increase of silicon-oxygen tetrahedron (SiO_4^{4-}) in the Bekenu series causes the formation of monosilicic acid (H_4SiO_4) via hydrolysis, where one mole of SiO_4^{4-} reacts with four moles of water to produce another four moles of OH^- (Antonangelo et al., 2017; Sahebi et al., 2015; Sugama & Pyatina, 2015).

Compared with soil only without any amendment (T0), the increased pH also resulted in reduced exchangeable acidity and Al^{3+} in the soils amended with SiMn slag and chemical fertilisers (T3, T4, T5, and T6) (Figures 4c and 4d). For example, the exchangeable acidity in T3, T4, T5, and T6 had significantly decreased to a range of 0.21 to 0.29 $cmol_{(+)}\ kg^{-1}$ compared with T0 at 1.00 $cmol_{(+)}\ kg^{-1}$ (Figure 4c). Furthermore, compared with the exchangeable Al^{3+} at 0.79 $cmol_{(+)}\ kg^{-1}$ (T0), the amendments in T3, T4, T5, and T6 significantly reduced soil exchangeable Al^{3+} to values ranging from 0.05 $cmol_{(+)}\ kg^{-1}$ to 0.14 $cmol_{(+)}\ kg^{-1}$ (Figure 4d).

The reduced soil exchangeable acidity and Al^{3+} are related to the alkalinity of SiMn slag, which is demonstrated by the significantly higher pH of SiMn slag (T1) in Figures 4a and 4b. The value of exchangeable Al^{3+} below the Al toxicity threshold of 0.8 $cmol_{(+)}\ kg^{-1}$ was due to the transformation of active exchangeable Al^{3+} in the soil solution into insoluble Al hydroxide ($Al(OH)_3$) via precipitation (Moir & Moot, 2014; Yerima et al., 2020). In addition, the impaired Al hydrolysis was due to a significant increase in soil pH, where the continuous dissolution of SiMn slag can increase the pH buffering capacity of the Bekenu series to prevent a drastic increase in active H^+ in the soil solution over time (Chong et al., 2022; Pavlů et al., 2021). The reaction of $Al(OH)_3$ with Si released from

SiMn slag leads to the formation of stable aluminium silicate ($Al_2O_3 \cdot 6SiO_2$) to prevent the reverse reaction of $Al(OH)_3$ into toxic Al^{3+}, which can further impair the production of H^+ via Al hydrolysis (Jo et al., 2022).

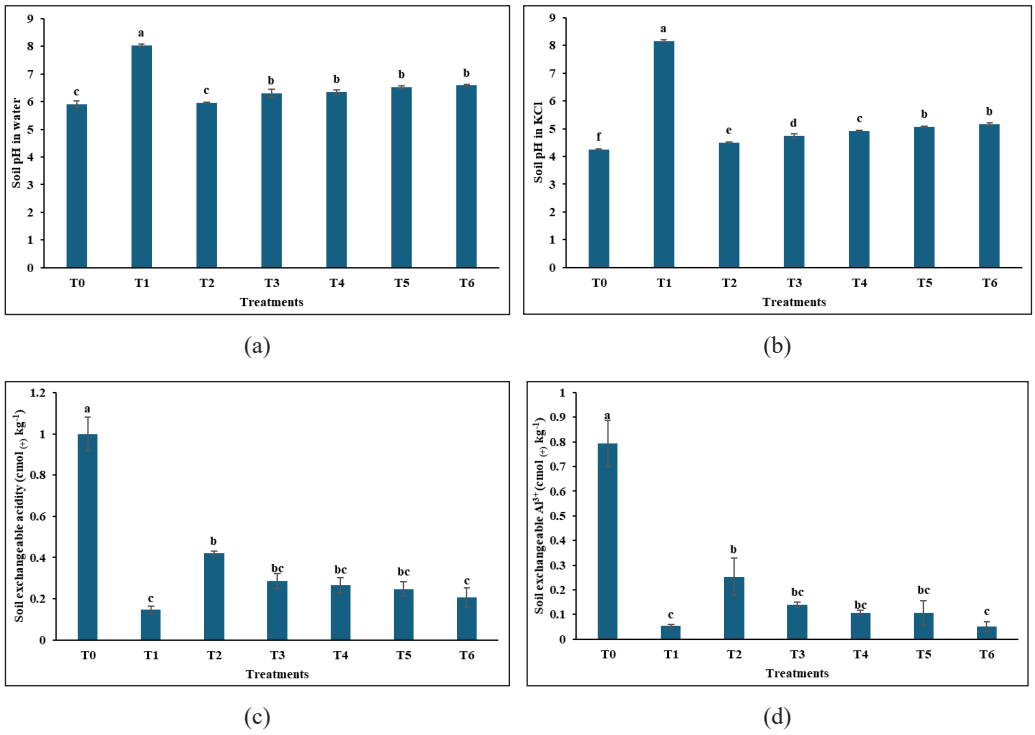


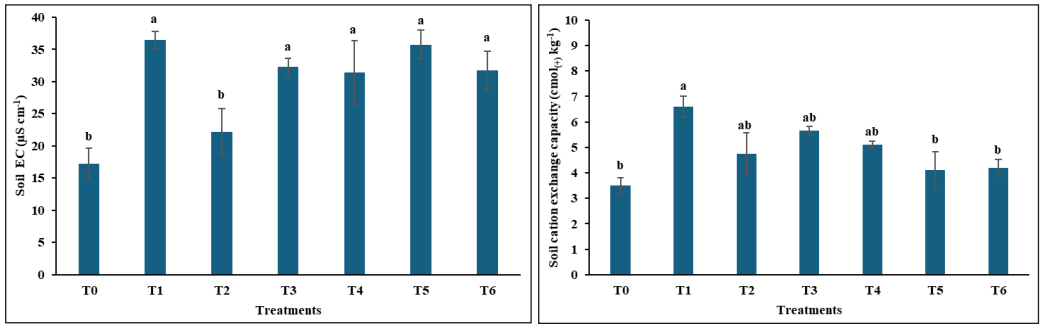
Figure 4. Soil (a) pH in water; (b) pH in potassium chloride; (c) exchangeable acidity; and (d) exchangeable aluminium in relation to treatments over thirty days of leaching. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates
Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF

Co-Application of Silico-Manganese Slag and Chemical Fertilisers on Soil Electrical Conductivity, Cation Exchange Capacity, and Nutrient Availability After Thirty Days of Leaching

In Figure 5a, the EC significantly increased in the soils added with SiMn slag and chemical fertilisers (T3, T4, T5, and T6) compared with soil only (T0) due to the presence of higher exchangeable K^+, Ca^{2+}, and Mg^{2+} in the SiMn slag, as demonstrated in T1. The significant increase in soil EC is related to the increased concentrations of soluble salts such as Na^+, K^+, Ca^{2+}, Mg^{2+}, CO_3^{2-}, and NO_3^- (Gondek *et al.*, 2020). Notably, regardless of treatment, the soil EC did not exceed the salinity threshold of 4000 µS cm^{-1}. Therefore, this finding suggests that the application rates of SiMn slag

used for the acidic soils in this study will not affect nutrient availability, microorganism activities, and crop growth (USDA, 2011).

Although the cation exchange capacity (CEC) of SiMn slag (T1) was significantly higher compared with soil only (T0), the inclusion of SiMn slag and/or chemical fertilisers (T2, T3, T4, T5, and T6) did not significantly improve the CEC of Bekenu series, regardless of the SiMn slag rate (Figure 5b). According to Aprile & Lorandi (2012) and Sdiri *et al.* (2012), the higher CEC can retain higher amounts of essential nutrients for optimum timely crop uptake, where the increased soil base saturation and pH can improve soil CEC via the increased number of negatively charged sites on the surface of alkaline materials added. Therefore, based on these findings, we concluded that the rates of SiMn slag should be further increased to improve the CEC of the Bekenu series.



(a) (b)

Figure 5. Soil (a) electrical conductivity and (b) cation exchange capacity in relation to treatments over thirty days of leaching. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF

After 30 days of leaching, exchangeable NH_4^+ in the soils with the sole application of chemical fertilisers only (T2) and the application of SiMn slag or/and chemical fertilisers (T3, T4, T5, and T6) was significantly higher compared with the soil only without any amendment (T0) (Figure 6a). This indicates that the significant improvement of NH_4^+ availability in the acidic soils was due to the application of chemical fertilisers because the SiMn slag (T1) had significantly lower exchangeable NH_4^+ compared with T0. This finding indicates that additional N source fertilisers will be needed to meet crop N requirements.

Interestingly, the combined use of SiMn slag and chemical fertilisers did not significantly affect soil available NO_3^- after 30 days of leaching (Figure 6b). This could be explained by the reduced nitrification, where the higher exchangeable NH_4^+ in the

soils following the incorporation of SiMn slag and chemical fertilisers, because the application of urea in T2, T4, T5, and T6 did not show a clear increasing trend in NO_3^-. Besides, the insignificant increase of soil available NO_3^- could be speculated by the high mobility of NO_3^- in soils (Gao et al., 2019; Zhang et al., 2020). The negatively charged sites on the soil colloids tend to repel NO_3^- (Lehmann & Schroth, 2003). Ng et al. (2024a) and Ng et al. (2021) also reported that the increased soil CEC retained a higher amount of exchangeable NH_4^+ to prevent nitrification. However, the use of SiMn slag did not significantly improve soil CEC; thus, the increase in pH is the dominant factor which prevents nitrification rather than CEC. Furthermore, SiMn slag has low inorganic N contents for both NH_4^+ and NO_3^-, thus the addition of nitrogen-based fertilisers is essential to improve soil N availability.

Similar with the total amount of P leached over 30 days of leaching (Figure 2c), the insignificant differences of soil available P (Figure 6c) following application of SiMn slag could be due to the low inherent P content in SiMn slag and the diminished effects by Al^{3+}, Fe^{2+} in the acidic soils (T0) as well as Ca^{2+} and Mg^{2+} in the soils with the inclusion of SiMn slag. Additionally, the sorbed P on Fe minerals was mobilised by Si released from the SiMn slag, potentially increasing P availability in the soil. For example, Ng et al. (2024b) and Schaller et al. (2019) claimed that Si has a higher affinity for binding with soil Fe minerals compared with P, where the P mobilisation positively correlates with increasing Si availability. Conversely, the insignificant differences in available P among the treatments could be due to the low dissolution of SiMn slag to release Si and insufficient application rates of SiMn slag used for P mobilisation in this soil leaching experiment.

At the end of the leaching experiment, the soil exchangeable Ca^{2+} significantly increased in the soils amended with SiMn slag or/and chemical fertilisers (T3, T4, T5, and T6) to a range of 1.42 mg kg^{-1} to 2.93 mg kg^{-1} compared with the soil only (T0) at 0.04 mg kg^{-1} (Figure 6d). Based on Figure 6e, soil exchangeable Mg^{2+} for T3 (0.31 mg kg^{-1}), T4 (0.38 mg kg^{-1}), T5 (0.47 mg kg^{-1}), and T6 (0.54 mg kg^{-1}) significantly increased compared with T0 (0.08 mg kg^{-1}) after 30 days of leaching. The increase in soil exchangeable Ca^{2+} and Mg^{2+} was because of the considerable inherent contents of the CaO and magnesium oxide (MgO) in the SiMn slag (Table 2). This finding is further supported by findings which reported that the SiMn slag has considerable contents of CaO and MgO, ranging from 9.24% to 29.10% and 3.70% to 6.91%, respectively (Cheng et al., 2023; Criado et al., 2018; Navarro et al., 2020; Navarro et al., 2017; Wang et al., 2017b). Therefore, we concluded that the use of SiMg slag can increase the base saturations in acidic soils to immobilise H^+ and mitigate Al and Fe hydrolysis to improve soil pH over time.

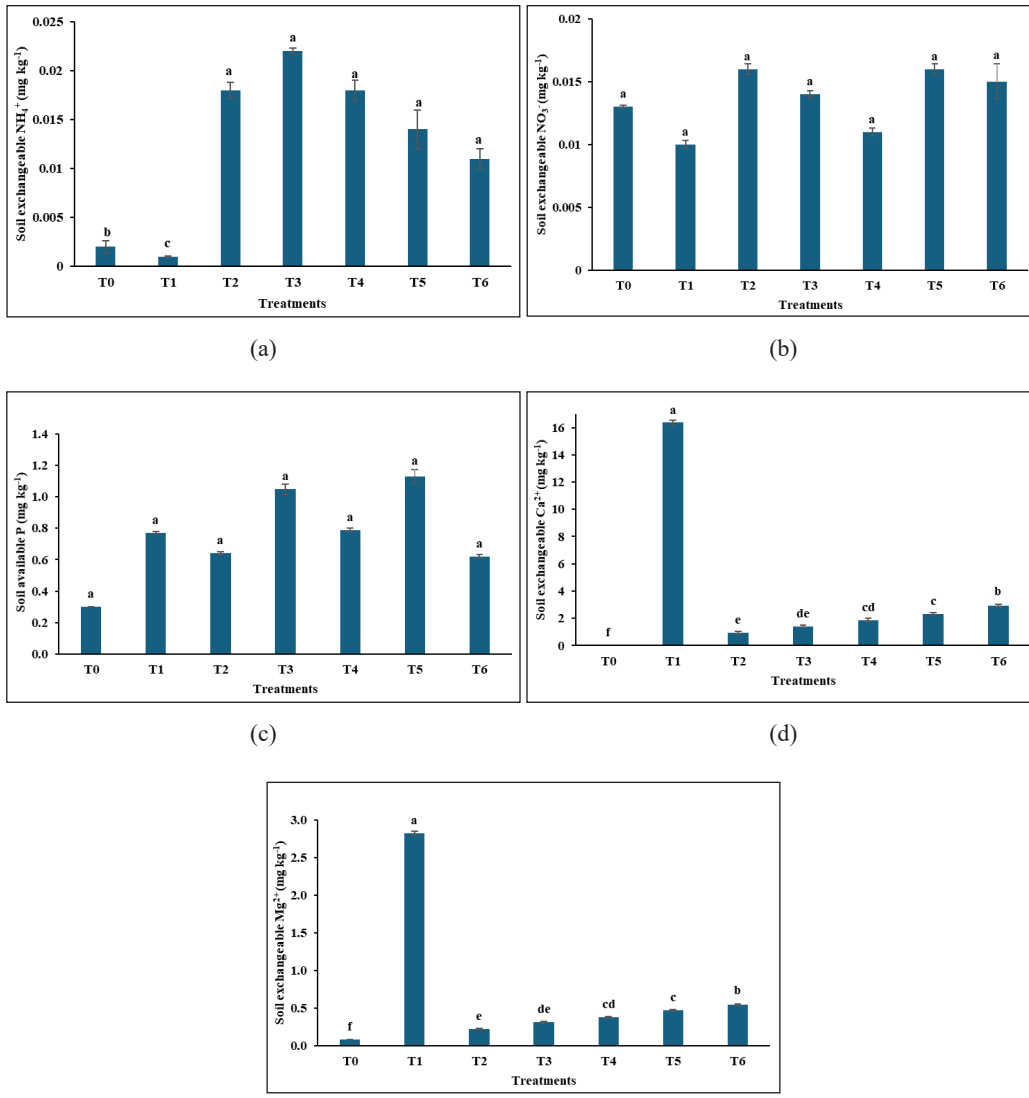


Figure 6. Soil (a) ammonium; (b) nitrate; (c) phosphorus; (d) calcium; and (e) magnesium in relation to treatments over thirty days of leaching. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. Different letters indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF

CONCLUSION

Co-applying SiMn slag and chemical fertilisers can improve soil pH, EC, exchangeable NH_4^+, Ca^{2+}, and Mg^{2+} in addition to suppressing exchangeable acidity and Al^{3+} of the

Bekenu series because of the alkalinity and liming effects (Ca^{2+} and Mg^{2+}) of SiMn slag. After 30 days of leaching, the higher amounts of NH_4^+, NO_3^-, K^+, Ca^{2+}, and Mg^{2+} leached from the amended soils are reflected by higher pH and EC of the leachates. This finding indicates that our approach did not mitigate nutrient leaching but rather improved the nutrient availability in the soil solution for rapid crop uptake. Based on our findings, the most suitable combinations are T5 (50% SiMn slag + 50% CF) and T6 (25% SiMn slag + 75% CF) per kilogram of soil because of their consistent effects on improving soil pH, exchangeable NH_4^+, Ca^{2+}, Mg^{2+}, and EC by suppressing exchangeable acidity and Al^{3+}. The use of SiMn slag as a scheduled industrial waste provides fundamental information to determine the effects of the suitable combinations uncovered by this present study on nutrient leaching and retention in the tropical acidic soils. Further pot and field research involving maize plants for revealing soil-plant interactions is recommended to further validate if the application of SiMn slag can reduce fertiliser dosage in the maize farming system.

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Photoperiod-specific LED Supplementation Enhances Growth Performance and Alters Biomass Allocation in Mustard Greens (*Brassica Juncea* L.) under Controlled Environment Agriculture

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ABSTRACT

Optimising supplemental lighting protocols for tropical controlled environment agriculture remains challenging due to limited photoperiod-specific research. This study investigated LED timing effects on mustard greens (*Brassica juncea* L.) growth under Vietnamese greenhouse conditions. Four treatments were evaluated: LED_0 (control), LED_18-22 pm (evening supplementation), LED_22 pm - 2 am (night supplementation), and LED_2-6 am (early morning supplementation). Custom LED modules with 50W Chip-on-Board technology provided 60-70 μmol.m^{-2}.s^{-1} PPFD at 0.5m distance. Plants were grown in commercial Tribat soil with automated irrigation maintaining 70-80% moisture. Morphological parameters, SPAD chlorophyll index, and biomass allocation were assessed over 42 days. Evening LED supplementation (18-22 pm) demonstrated superior performance, achieving a 70.7% increase in plant height (61.64 vs 36.10 cm), a 58.0% enhancement in total fresh weight (20.65 vs 13.07 g), and a 26.2% improvement in leaf number compared to controls. However, this treatment exhibited morphological trade-offs with a 11.8% reduction in leaf width and 18.2% decrease in leaf length. Biomass allocation shifted dramatically from 90.15% leaf allocation in controls to 77.06% under evening supplementation, with stem allocation increasing from 1.92% to 17.33%. SPAD analysis revealed timing-dependent chlorophyll responses, with early enhancement under evening treatment and superior mid-season development under early morning supplementation. These findings establish that 4-hour evening LED supplementation optimally synchronises with *Brassica juncea* circadian

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rhythms, providing an energy-efficient enhancement of productivity while highlighting critical morphological consequences for commercial applications in tropical controlled-environment agriculture.

Keywords: Brassica juncea, biomass allocation, controlled environment agriculture, LED timing, photoperiod optimisation, tropical greenhouse

INTRODUCTION

With rapid urbanisation and growing food security demands, controlled environment agriculture (CEA) has emerged as a critical solution for sustainable crop production, particularly where arable land is limited (Acero, 2013; Rouphael et al., 2010). Indoor farming systems utilising artificial lighting enable year-round production of high-quality crops with minimal environmental impact (Bian et al., 2015; Dou et al., 2018). However, low natural daily light integrals (DLIs) during darker months, particularly in higher-latitude regions (5-15 $mol \cdot m^{-2} \cdot d^{-1}$), and seasonal limitations in tropical regions during rainy seasons necessitate optimised supplemental lighting systems (Faunce, 2012; Pham et al., 2024).

Light-emitting diodes (LEDs) have revolutionised agricultural lighting by offering superior energy efficiency, precise wavelength control, and reduced heat generation compared to traditional systems (Cocetta et al., 2017; Mitchell et al., 1991). The spectral tunability of LEDs enables targeted manipulation of photoreceptor systems, particularly through red (660-670 nm) and blue (440-460 nm) wavelengths that demonstrate high photosynthetic efficiency (Massa et al., 2008; Olle & Viršile, 2013). Photosynthesis is highly dependent on photosynthetically active radiation (PAR, 400-700 nm), with blue light crucial for chlorophyll formation and stomatal regulation, while red light efficiently drives photosynthetic energy conversion and biomass accumulation (Acero, 2013; Terashima et al., 2009).

Brassica species, including leafy Brassica vegetables, are particularly valuable due to their rapid growth cycles, high responsiveness to light quality modifications, and complex secondary metabolite pathways contributing to health-promoting properties (Kyriacou et al., 2016). These crops demonstrate optimal growth under controlled conditions with PPFD ranges of 220-440 $\mu mol.m^{-2}.s^{-1}$, making them ideal candidates for LED-based production systems (Jones-Baumgardt et al., 2019; Samuolienė et al., 2013).

Recent research has expanded beyond traditional red-blue LED combinations to explore effects of broader spectral ranges, with studies showing that light intensity significantly influences morphology and biomass accumulation (Brazaitytė et al., 2019; Gerovac et al., 2016). However, significant knowledge gaps remain regarding optimal LED strategies for enhancing both productivity and phytochemical content in leafy Brassica crops, particularly under practical greenhouse conditions (Podsędek et al., 2023). Despite lighting

costs accounting for 40-80% of total production expenses, comprehensive investigations of spectral effects on primary metabolites, carotenoids, chlorophylls, and antioxidant compounds remain limited (Podsędek et al., 2023; Semenova et al., 2022).

Based on 28 recently different collected studies, it shows that only 3 experiments were conducted in temperate regions, with the majority being conducted in temperate and subtropical regions on seedlings (microgreens) deployed in strictly controlled environments such as growth chambers or laboratories, where light, temperature and humidity are artificially regulated, commonly using LED lights or other artificial light sources. Supplemental experiments with LED lighting at different times throughout the night when grown on clean soil under greenhouse conditions have not been found. The critical knowledge gap in photoperiod-specific LED supplementation for tropical microgreen production under practical greenhouse conditions necessitates this investigation. While existing studies predominantly focus on controlled laboratory environments in temperate regions, the unique challenges of tropical agriculture, including seasonal variations in natural light intensity, high humidity conditions, and economic constraints of small-scale farmers, remain inadequately addressed. Furthermore, the optimisation of LED timing protocols specifically for *Brassica juncea* cultivars adapted to Southeast Asian growing conditions has not been systematically evaluated, despite the species' significant commercial potential and nutritional value in regional markets. The substantial economic implications of lighting costs (40-80% of production expenses) combined with limited understanding of circadian rhythm synchronisation under tropical photoperiods create an urgent need for evidence-based LED supplementation strategies. Recent studies have begun to address this photoperiod-timing gap directly. A hydroponic trial comparing the same 4-hour supplemental windows (18:00-22:00, 22:00-02:00, and 02:00-06:00) in mustard greens found that pre-dawn (02:00-06:00) lighting produced the greatest gains in SPAD index, leaf number, plant height, and leaf width, yielding 44% higher fresh weight and 52% higher dry biomass than the control, with the highest leaf-to-total biomass ratio and lowest stem ratio (Duong et al., 2026). Separately, a photoperiod-duration trial (8-24 hours) on *Brassica* microgreens showed that short photoperiods (8 hours) promoted hypocotyl elongation and fresh weight, whereas continuous 24-hour illumination suppressed growth most severely (Vaštakaitė-Kairienė et al., 2022). These findings, however, were obtained in hydroponic or short-cycle microgreen systems rather than in soil-grown mustard greens over a complete production cycle, leaving the comparative efficacy of specific nocturnal timing windows under tropical greenhouse soil culture still unresolved. Therefore, this study aims to bridge these critical gaps by evaluating photoperiod-specific LED timing effects on growth performance, biomass allocation, and physiological responses of mustard greens under realistic Vietnamese greenhouse conditions, providing essential relevance for sustainable intensification of tropical controlled environment agriculture systems in the future.

MATERIALS AND METHODS

Experimental Conditions

The greenhouse experiment was conducted at Kien Giang University, Chau Thanh district, Kien Giang province, Vietnam (9°54'49.05"N, 105°8'33.7992"E) from March 3rd to April 14th, 2025. Environmental parameters were continuously monitored using calibrated instruments: an Indoor Digital Temperature Humidity Meter (Beurer, Type: HM 16, Art.-Nr: 679.15) for air humidity and temperature measurements, and a Photosynthesis Light Quantum Meter (TES 1339P) for recording Photosynthetic Photon Flux Density (PPFD). The mean environmental conditions inside and outside the greenhouse were recorded as follows: PPFD (208/750 µmol.m^{-2}.s^{-1}), temperature (30/32 °C), and humidity (71/61%), respectively.

Growth Medium and Lighting System

Growth Medium

The growing substrate consisted of commercially available clean soil (Tribat soil, Sai Gon Xanh company, Vietnam) specifically formulated to provide adequate nutrition for mustard cultivation. The soil was filled to a depth of 2 dm in containers measuring 1 m width × 10 m length. The nutrient composition of Tribat soil (dry weight basis) included: humus (15%), organic matter (25%), total nitrogen (1%), potassium K_2O (0.73%), total phosphate P_2O_5 (0.3%), cation exchange capacity (CEC: 45 meq.100g^{-1}), pH 6.5, and salinity 1298 ppm. Additional micronutrients such as Mg, Mn, Zn, B, Cu, Mo, and chelated iron (Fe-EDTA-13) were present, though specific concentrations were not disclosed by the manufacturer.

LED Lighting System

A custom LED module was designed and assembled by the research team based on preliminary trials. Each module comprised four Chip-on-Board (CoB) LEDs arranged with one pink light centrally positioned and three white lights surrounding it (Figure 1, left and middle), with power consumption about 800Wh. All commercially available generic full-

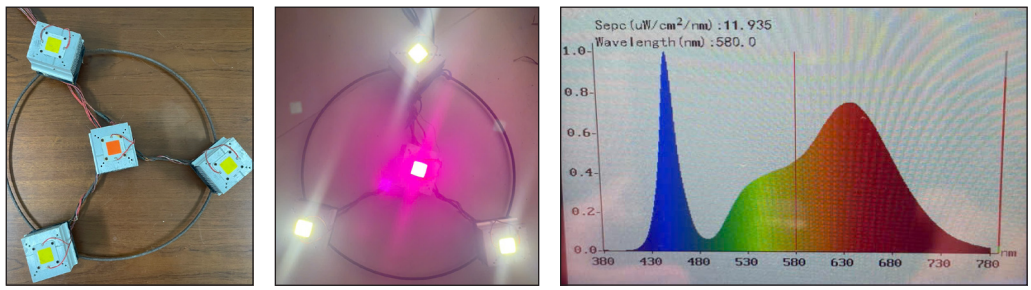


Figure 1. LED module: off (left), on (middle) and its spectral quality (right)

spectrum COB LED chips (46 × 40 mm package, model code 4640-4C30B) were sourced from China and shared identical specifications: voltage 12V-14V DC, power consumption 50 watts, luminous efficacy 90-120 LM/W, operating temperature range -20 °C to 60 °C, and spectral bandwidth 400-780 nm. COB modules were selected for their low cost, local availability, and ease of custom module fabrication for a multi-treatment timing experiment, which would have been cost-prohibitive with dedicated horticultural LED arrays.

Spectral quality was quantified using an OHSP-350BF Portable Spectral Irradiance Colourimeter (wavelength range: 380-780 nm), with an R:G:B ratio of 48:32:20 (Figure 1, right). Photosynthetic photon flux density (PPFD) of 60-70 $\mu mol.m^{-2}.s^{-1}$ was measured using a Photosynthesis Light Quantum Meter (TES-1339P) at a distance of 0.5 m from the centre of the LED module. The spacing between COB LEDs was 40.05 cm (white-to-white) and 23 cm (white-to-pink).

Experimental Design

The experiment followed a completely randomised design with three replications conducted under controlled greenhouse conditions. Four distinct LED lighting treatments were evaluated: LED_0 (control treatment with LED system off), LED_18-22 pm (LED illumination from 6:00 PM to 10:00 PM), LED_22 pm-2 am (LED illumination from 10:00 PM to 2:00 AM), and LED_2-6 am (LED illumination from 2:00 AM to 6:00 AM). Each treatment consisted of 3 pots with dimensions of 57 cm (length) × 36 cm (width) × 16 cm (height), resulting in a total of 12 experimental plots.

The LED modules were positioned to maintain a consistent distance of 0.5 m from the lamp to the leaf tips, as determined through pilot studies; the LED module height was adjusted (raised) as the plants grew. Supplemental lighting was initiated when mustard seedlings reached 7 days after germination. The on/off cycling of the LED modules for each treatment was automatically controlled using a digital timer switch (ATA, Model AT-20B). This programmable timer allowed multiple independent on/off switching cycles to be preset within a 24-hour period, with a minimum switching interval of 1 minute. Prior to the experiment, each timer was programmed and calibrated according to the designated photoperiod schedule for each treatment (LED_18-22h, LED_22h-2h, and LED_2-6h), ensuring that the LED modules were automatically activated and deactivated at the specified start and end times daily throughout the growth period. The control treatment (LED_0) was connected to the power supply but remained switched off for the entire experimental duration (Figure 2). Each timer unit was connected in series between the main power source and the corresponding LED module, and its accuracy was periodically verified against a reference clock to ensure consistency of the photoperiod treatments across.

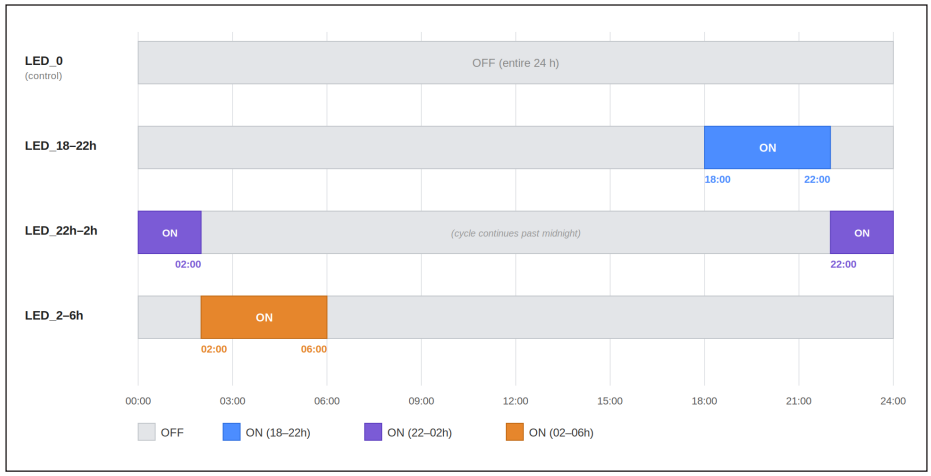


Figure 2. 24-hour LED on/off Schedule by treatment

Crop Establishment and Management

Seed Establishment

Mustard green seeds obtained from Vietnamese Dai Dia company, representing a tropical variety commonly cultivated in southern Vietnam, were directly sown onto the Tribat soil substrate at a seeding rate of 0.2 g per square meter. Soil moisture was maintained consistently at 70-80% throughout the experimental period using a garden hose irrigation system, with moisture levels monitored using a soil moisture tester (MD-15). Non-uniform seedlings were removed to achieve and maintain a uniform population density of 100 plants per square meter.

Data Collection and Measurements

Growth Parameters and Yield Assessment

Initial measurements commenced when mustard plants developed three fully expanded leaves (one week after light treatment initiation), followed by subsequent measurements at 5-day intervals. From each plot, 45 randomly selected and marked plants (15 plants per pot) were used for growth parameter collection. Morphological measurements included leaf length (measured from the tip of the longest leaf to the petiole), leaf width (at the widest point), and plant height (from ground level to the tip of the topmost leaf).

Yield assessment was conducted 42 days after seeding by flooding the pots to facilitate soil-root separation. Plant components (leaves, stems, and roots) were carefully separated and weighed to determine fresh yield parameters.

SPAD Index Measurement

Chlorophyll content was estimated using the SPAD-502Plus Chlorophyll Meter. The third fully expanded leaf from the top was randomly selected from ten plants in each plot. The final SPAD value represented the mean of 15 individual readings per plot.

Growth Rate Calculation

Absolute Growth Rate (AGR) was calculated using the following Equation 1 (Lalruatfeli, 2021):

$$AGR = (W_2 - W_1) / (T_2 - T_1) \ (g.day^{-1}) \quad [1]$$

where W_1 and W_2 represent dry matter weight at times T_1 and T_2, respectively.

Statistical Analysis

Data analysis was performed using Analysis of Variance (ANOVA) appropriate for a split-plot experimental design. Mean separation was conducted using Duncan's Multiple Range Test at a $P \leq 0.05$ significance level. All statistical analyses were executed using SPSS Version 20.0 software package.

RESULTS

Effect Of Various LED Lighting Timings on Mustard Green Growth

Based on the experimental data, LED supplemental lighting significantly enhanced mustard green growth compared to the control treatment. The LED_18-22 pm treatment demonstrated superior performance, achieving the highest final plant height of 61.64 cm at 42 days after sowing (DAS), representing a 70.7% increase over the control (36.10 cm) (Table 1). This treatment maintained consistent growth throughout the experimental period, with notable acceleration during periods 2-4. The LED_2-6 am treatment produced intermediate results (45.81 cm final height), while LED_22 pm-2 am showed the poorest performance among supplemental lighting treatments (32.85 cm). Growth rate analysis revealed that the LED_18-22 pm treatment exhibited the most balanced growth pattern, with reduced initial growth rate (1.02 cm·day^{-1}) but sustained performance in later periods. These findings suggest that evening LED supplementation (18-22 pm) optimally synchronises with the natural photoperiod and circadian rhythms of *Brassica juncea*, resulting in enhanced vegetative growth compared to other timing regimens or natural lighting conditions alone.

LED supplemental lighting exhibited contrasting effects on leaf width development in mustard greens compared to plant height responses. The control treatment (LED_0) achieved the greatest final leaf width of 8.28 cm at 42 DAS, significantly outperforming

Table 1
*Effect of various LED lighting timings on plant height (cm) of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Plant Height (cm)					Height Growth Rate (cm.day^{-1})			
	14 DAS	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**	**Period1**	**Period2**	**Period3**	**Period4**
LED_0	11.04b	21.37b	25.04b	31.10c	36.10c	1.08a	1.95a	1.41a	1.48a
LED_18-22 pm	15.49a	21.27b	36.10a	48.27a	61.64a	1.02a	0.82b	0.80b	1.00b
LED_22 pm-2 am	15.06a	22.42a	26.64b	29.52c	32.85c	1.11a	1.13b	0.85b	0.80b
LED_2-6 am	16.17a	23.90a	33.76a	40.01b	45.81b	0.60b	1.16b	0.61b	0.61b
Sig.	***	***	***	***	***	**	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: ** ($P < 0.01$), *** ($P < 0.001$). Days after sowing: DAS

the LED_18-22 pm treatment (7.30 cm), which showed a 11.8% reduction (Table 2). Early growth patterns revealed that LED_18-22 pm initially promoted leaf width expansion at 14 DAS (4.15 cm vs. 3.35 cm in control), but this advantage diminished over time, with slower growth rates observed in periods 2-4 (0.10, 0.06, and 0.09 cm·day^{-1}, respectively). The LED_22 pm-2 am treatment demonstrated the highest leaf width at 28 DAS (7.16 cm) with the most rapid expansion during period 2 (0.26 cm·day^{-1}), though data beyond this timepoint were unavailable. These findings suggest that while evening LED supplementation (18-22 pm) effectively promotes vertical growth, it may redirect metabolic resources away from lateral leaf expansion, potentially indicating a trade-off between height and leaf width development under artificial photoperiod manipulation in *Brassica juncea*.

LED supplemental lighting significantly influenced leaf length development in mustard greens, with the control treatment maintaining superior performance throughout the experimental period (Table 3). The LED_0 treatment achieved the highest final leaf length of 21.78 cm at 42 DAS, while LED_18-22 pm showed the poorest response with only 17.81 cm, representing an 18.2% reduction compared to the control. Despite initial advantages in early growth (11.33 cm at 14 DAS vs. 9.47 cm in the control), the LED_18-22 pm treatment exhibited dramatically reduced growth rates in later periods (0.20, 0.09, and 0.09 cm·day^{-1} for periods 2-4, respectively), compared to the control's consistent rates (0.47, 0.24, and 0.24 cm·day^{-1}). The LED_22 pm-2 am treatment performed comparably to the control (21.44 cm final length) with the highest growth rate during period 2 (0.58 cm·day^{-1}). These results demonstrate that evening LED supplementation (18-22 pm) creates a pronounced trade-off between vertical stem elongation and leaf length expansion, suggesting that while this photoperiod manipulation effectively promotes plant height, it simultaneously constrains leaf morphological development in *Brassica juncea*.

LED supplemental lighting significantly enhanced leaf number development in mustard greens, with LED_18-22 pm and LED_2-6 am treatments demonstrating superior performance compared to the control (Table 4). The LED_18-22 pm treatment achieved the highest leaf number at 35 and 42 DAS (10.289 and 10.378 leaves, respectively), representing a 26.2% and 20.0% increase over the control (8.156 and 8.644 leaves). The LED_2-6 am treatment produced the greatest final leaf count of 10.667 leaves at 42 DAS, showing a 23.4% improvement compared to the control. Notably, LED_18-22 pm exhibited an exceptional leaf production burst during period 2 (0.53 leaves·day^{-1}), the highest rate among all treatments and periods, followed by dramatic reductions in later periods (0.03 and 0.01 leaves·day^{-1}). The LED_2-6 am treatment demonstrated the most consistent leaf development pattern with sustained growth rates throughout the experimental period (0.39, 0.11, and 0.17 leaves·day^{-1} for periods 2-4). These findings indicate that both evening (18-22 pm) and early morning (2-6 am) LED supplementation effectively stimulate leaf initiation and development, with evening lighting promoting rapid early leaf emergence while early morning lighting maintains more sustained leaf production in *Brassica juncea*.

Table 2
*Effect of various LED lighting timings on leaf width of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Width (cm)					Leaf Width Growth Rate (cm)			
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	Period1	Period2	Period3	Period4
LED_0	3.35c	5.47b	6.79 b	7.49a	8.28a	0.30a	0.19b	0.10a	0.11a
LED_18-22 pm	4.15a	5.59b	6.27c	6.69b	7.30b	0.21b	0.10c	0.06b	0.09b
LED_22 pm-2 am	3.69b	5.34b	7.16 a	-	-	0.24b	0.26a	-	-
LED_2-6 am	3.82b	5.86a	6.87 ab	-	-	0.29a	0.14bc	-	-
Sig.	***	**	***	***	***	***	***	***	*

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2am and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$). Days after sowing: DAS. "-" indicates data missing due to a field data-recording oversight

Table 3
*Effect of various LED lighting timings on leaf length of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Length (cm)					Leaf Length Growth Rate (cm.day^{-1})			
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	Period1	Period2	Period3	Period4
LED_0	9.47b	15.13	18.44a	20.12a	21.78a	0.81	0.47a	0.24a	0.24a
LED_18-22 pm	11.33a	15.16	16.56b	17.21c	17.81c	0.55	0.20c	0.09c	0.09c
LED_22 pm-2 am	10.70a	14.83	18.92a	20.04a	21.44a	0.59	0.58a	0.16b	0.20ab
LED_2-6 am	11.00a	18.27	17.94a	19.02b	20.27b	0.66	0.33b	0.15b	0.18b
Sig.	***	ns	***	***	***	**	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am and 2- 6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: ** ($P < 0.01$), *** ($P < 0.001$), ns (not significant). Days after sowing: DAS

Table 4
*Effect of various LED lighting timings on leaf number of mustard greens (*Brassica juncea *L.)*

Treatment	Mean Leaf Number					Leaf Number Growth Rate (Leaf.day^{-1})			
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS	Period1	Period2	Period3	Period4
LED_0	4.93b	6.42a	7.24d	8.156c	8.644b	0.21a	0.12c	0.13a	0.07b
LED_18-22 pm	4.89b	6.40a	10.11a	10.289a	10.378a	0.22a	0.53a	0.03b	0.01c
LED_22 pm-2 am	4.78b	5.56c	7.80c	8.178c	8.489b	0.11b	0.32b	0.05b	0.04bc
LED_2-6 am	5.60a	5.98b	8.69b	9.489b	10.667a	0.05b	0.39b	0.11a	0.17a
Sig.	***	***	***	***	***	***	***	***	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: *** ($P < 0.001$). Days after sowing: DAS

Effect of Various LED Lighting Timings on SPAD Index of Mustard Green

LED supplemental lighting demonstrated significant effects on chlorophyll content (SPAD index) in mustard greens during early and mid-growth stages. The LED_18-22 pm treatment exhibited the highest SPAD value at 14 DAS (30.07), representing a 12.1% increase over the control (26.83), indicating enhanced early chlorophyll synthesis under evening LED supplementation (Table 5). However, the most pronounced response occurred with LED_2-6 am treatment at 35 DAS, achieving a SPAD value of 35.83, which was significantly higher than all other treatments and represented a 13.9% increase compared to the control (31.45). This delayed chlorophyll enhancement suggests that early morning LED supplementation optimally synchronises with natural photosynthetic processes during the critical reproductive transition period. Interestingly, SPAD differences among treatments became non-significant at the final harvest (42 DAS), with all treatments converging to similar chlorophyll levels (32.14-33.24). These findings indicate that LED photoperiod manipulation primarily influences chlorophyll development timing rather than final chlorophyll accumulation, with early morning supplementation (2-6 am) providing the most substantial mid-season chlorophyll enhancement in *Brassica juncea*.

Effect of Various LED Lighting Timings on Yield and Biomass Allocation of Mustard Green Growth

LED supplemental lighting significantly enhanced total biomass production and altered biomass allocation patterns in mustard greens (Table 6). The LED_18-22 pm treatment achieved the highest total fresh weight of 20.65 g, representing a 58.0% increase over the control (13.07 g), primarily attributed to superior leaf (15.68 g vs. 11.74 g) and stem development (4.19 g vs. 0.26 g). This treatment demonstrated a dramatic shift in biomass allocation, with stem biomass allocation increasing from 1.92% in the control to 17.33%, while leaf allocation decreased from 90.15% to 77.06%. The LED_22 pm-2 am and LED_2-6 am treatments showed even more pronounced stem allocation increases (20.94% and 21.73%, respectively) but with reduced total biomass production. Root biomass allocation significantly decreased under LED supplementation, particularly in the LED_2-6 am treatment (6.54% vs. 9.22% in control). These findings indicate that evening LED supplementation (18-22 pm) optimally balances enhanced total biomass production with modified architectural development, promoting stem elongation while maintaining substantial leaf biomass for photosynthetic capacity in *Brassica juncea*, suggesting improved light capture efficiency through altered morphological plasticity.

DISCUSSION

The present study demonstrates that LED photoperiod timing significantly influences growth patterns and morphological development in *Brassica juncea*, with results that

Table 5
*Effect of various LED lighting timings on SPAD of mustard greens (*Brassica juncea *L.)*

Treatment	Mean SPAD Index				
	14 DAS	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**
LED_0	26.83c	29.14	30.65	31.45b	32.62
LED_18-22 pm	30.07a	29.40	30.89	32.62b	32.14
LED_22 pm-2 am	28.28b	29.67	30.85	31.11b	33.06
LED_2-6 am	27.17c	29.60	30.48	35.83a	33.24
Sig.	***	ns	ns	***	ns

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: *** ($P < 0.001$), ns (not significant). Days after sowing: DAS

Table 6
*Effect of various LED lighting timings on yield components and the ratio of biomass allocation of mustard greens (*Brassica juncea *L.)*

Treatment	Fresh Weight (g)				Fresh Biomass Allocation (%)		
	Root	**Stem**	**Leaf**	**Total**	**Root**	**Stem**	**leaf**
LED_0	1.24ab	0.26b	11.74ab	13.07b	9.22a	1.92b	90.15a
LED_18-22 pm	1.48a	4.19a	15.68a	20.65a	8.50ab	17.33a	77.06b
LED_22 pm-2 am	0.76bc	2.48a	9.55bc	12.65b	7.75ab	20.94a	77.13b
LED_2-6 am	0.66c	2.55a	6.86c	10.07b	6.54b	21.73a	71.73b
Sig.	**	*	**	**	*	**	***

Note. LED_0, LED_18-22 pm, LED_22 pm-2 am and LED_2-6 am indicate no additional LED (control), LED lighting time from 18 to 22 pm, 22 pm to 2 am, and 2-6 am, respectively. Different letters within columns indicate significant differences ($P \leq 0.05$, Duncan's test). Significance levels: * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$)

both corroborate and extend previous findings on photoperiod-specific optimisation for Brassica species. The superior performance of the LED_18-22 pm treatment validates the critical importance of timing windows identified in recent literature while revealing novel trade-offs in resource allocation.

Interpreting Growth Responses at Low Supplemental PPFD

The PPFD delivered by the LED modules (60-70 μmol.m^{-2}.s^{-1} at 0.5 m) is well below the light-saturation range reported for Brassicaceae under continuous daytime supplementation (100-300 μmol.m^{-2}.s^{-1}), but matches independent measurements from our own group using the same CoB LED system (47 μmol.m^{-2}.s^{-1} in Duong et al. (2024) and 30.50-158.38 μmol.m^{-2}.s^{-1} across the canopy in Duong et al. (2026)), confirming this is the true irradiance rather than measurement error.

Given this sub-saturating PPFD, we propose that the pronounced morphological responses - notably the 70.7% increase in plant height under LED_18-22 pm despite modest leaf-dimension gains - are not primarily driven by enhanced photosynthetic carbon assimilation. Since all treatments were delivered as discrete nighttime pulses rather than continuous daytime light, a low-fluence photomorphogenic signal mediated by phytochrome/cryptochrome is more plausible, consistent with classical night-break and photoperiod-extension responses in Brassica species. Under this view, the LED pulses act as a circadian/photoperiod cue - shifting perceived night length and redirecting assimilate partitioning toward stem elongation - rather than as a direct carbon source. This aligns with our companion study (Duong et al., 2024), where exposure timing and duration, not PPFD magnitude, principally determined growth and flowering under similarly low irradiance (47-58 μmol.m^{-2}.s^{-1}). The decoupling between height enhancement (70.7%) and biomass gain (58.0%) further supports a photomorphogenic rather than purely photosynthetic explanation, since a pure carbon-assimilation effect should scale more proportionally with biomass than elongation.

Photoperiod Duration and Biomass Enhancement

The 70.7% increase in plant height and 58.0% enhancement in total fresh weight under LED_18-22 pm treatment strongly supports (Duong et al., 2024) findings that 4-hour evening LED supplementation (creating 16-hour total photoperiods) optimises mustard green growth parameters. This performance significantly exceeds the negative effects observed with 6-hour supplementation in their study, confirming the existence of an optimal threshold for supplemental lighting duration. Our results align with Liu et al. (2022)'s demonstration that 14-hour photoperiods maximised biomass in cabbage and Chinese kale microgreens, suggesting that 16-hour total duration represents near-optimal conditions for *Brassica juncea*. The consistent growth rates maintained throughout periods

2-4 under LED_18-22 pm treatment contrast with the declining performance observed in longer photoperiod treatments, supporting (Pham et al., 2024) observations that extended LED durations can shift from beneficial to detrimental effects. This temporal stability validates the hypothesis that evening supplementation optimally synchronises with natural circadian rhythms without disrupting essential dark-period processes. Interestingly, our finding that LED_18-22 pm outperformed LED_2-6 am for total biomass contrasts with a recent hydroponic trial using an identical 4-hour treatment structure, in which the pre-dawn (02:00-06:00) window, rather than the evening window, produced the highest fresh and dry biomass along with the greatest leaf-to-stem ratio (Duong et al., 2026). This divergence may reflect fundamental differences between soil-based and hydroponic nutrient delivery, or between the specific mustard cultivars used, and suggests that the optimal LED timing window may not be universally transferable across production systems. Further comparative trials using matched cultivars and growing media are needed to determine whether soil-grown *Brassica juncea* responds differently to nocturnal LED timing than hydroponically grown counterparts.

Light Intensity Effects and Morphological Responses

The enhanced early chlorophyll synthesis under LED_18-22 pm treatment (12.1% increase at 14 DAS) corroborates findings by (Liu et al., 2022) that optimal light intensity promotes higher SPAD values in Brassica species. However, the convergence of chlorophyll levels at harvest suggests that photoperiod timing primarily influences synthesis kinetics rather than final accumulation, extending (Singh et al., 2020) observations on SPAD meter applications for monitoring photosynthetic responses. The pronounced trade-off between vertical growth enhancement and leaf dimension reduction provides new insights into the morphological plasticity described by (Gerovac et al., 2016). While their study showed that higher light intensity generally decreases plant height in microgreens, our results demonstrate that timing-specific supplementation can reverse this relationship, achieving both height enhancement and substantial biomass increases. The 11.8% reduction in leaf width and 18.2% decrease in leaf length under LED_18-22 pm treatment suggest resource reallocation mechanisms similar to those observed under varying light intensities, but with timing-dependent modulation.

Biomass Allocation Patterns and Architectural Changes

The dramatic shift in biomass allocation from 90.15% leaf allocation in controls to 77.06% under LED_18-22 pm treatment, with stem allocation increasing from 1.92% to 17.33%, provides quantitative validation of Pham et al. (2024) observations that LED duration influences biomass partitioning. This reallocation pattern exceeds the magnitude reported in their study, likely due to the specific timing optimisation achieved with evening

supplementation. The even more pronounced stem allocation under LED_22 pm-2 am (20.94%) and LED_2-6 am (21.73%) treatments, despite reduced total biomass, suggests that night-time supplementation may trigger different physiological responses than evening extension. These allocation changes align with findings that biomass partitioning shifts from leaves to stems at later developmental stages (Singh et al., 2013), but our results indicate that LED timing can accelerate or modify this natural progression. The maintained photosynthetic capacity despite reduced leaf allocation suggests improved light capture efficiency through architectural modification, supporting the morphological plasticity hypothesis proposed in recent literature.

Chlorophyll Dynamics and Photosynthetic Optimisation

The temporal SPAD responses reveal sophisticated photoperiod-dependent metabolic programming previously unrecognised in Brassica LED studies. The early enhancement under LED_18-22 pm followed by superior mid-season response of LED_2-6 am treatment (13.9% increase at 35 DAS) demonstrates that different timing windows optimise distinct phases of chlorophyll development. This finding extends observations on spectral effects to include temporal optimisation, suggesting that both when and how LED supplementation is applied critically influences photosynthetic capacity (Li et al., 2019; Wu et al., 2013). The convergence of SPAD values at harvest, despite significant mid-season differences, corroborates Andrew (2023) observations that chlorophyll responds dynamically to light environments while maintaining homeostatic regulation. This temporal pattern suggests opportunities for stage-specific LED management strategies that optimise photosynthetic efficiency throughout the growing cycle.

Leaf Development and Production Efficiency

The exceptional leaf production burst under LED_18-22 pm treatment (0.53 leaves·day^{-1} from 14 to 21 after sowing), followed by a dramatic reduction, provides new insights into the timing-dependent responses reported by Duong et al. (2024). This pattern suggests that evening LED supplementation may trigger accelerated developmental programming that front-loads leaf production but potentially limits sustained growth capacity. The contrast with LED_2-6 am treatment's more consistent leaf production (0.39, 0.11, and 0.17 leaves·day^{-1} for periods 2-4) indicates alternative optimisation strategies for different production objectives. These findings extend observations on continuous lighting effects to reveal that timing-specific supplementation can achieve similar yield enhancement with potentially greater energy efficiency (Shibaeva et al., 2022). The 26.2% increase in leaf number under LED_18-22 pm treatment at 35 DAS approaches the yield improvements reported for continuous LED systems while using significantly less energy input.

Integration with Mustard Green Production Systems

The results provide important validation and extension of recent microgreen research. The enhanced fresh weight accumulation under LED_18-22 pm treatment (15.68 g vs. 11.74 g in controls) aligns with Gerovac et al. (2016) findings that appropriate light management increases biomass in mustard greens. However, our study demonstrates that timing optimisation can achieve these benefits while maintaining larger plant architecture, suggesting applications beyond microgreen production. The shorter harvest time implications identified by Duong et al. (2024) are supported by our growth rate data, particularly the accelerated early development under evening LED supplementation. This acceleration, combined with the maintained quality parameters, suggests commercial potential for LED timing optimisation in various Brassica production systems.

Implications for Controlled Environment Agriculture

The study's findings have significant implications for precision lighting strategies in controlled environment agriculture. The optimal 4-hour evening supplementation window identified validates recent industry trends toward targeted photoperiod management rather than continuous supplementation. The substantial yield improvements (58.0% increase in total biomass) achieved with relatively modest energy inputs support the economic viability of timing-specific LED strategies. However, the observed trade-offs between height enhancement and leaf expansion suggest that production objectives must guide LED timing selection. For applications prioritising leaf quality and surface area, alternative timing strategies or hybrid approaches may be more appropriate than the optimal evening supplementation identified for biomass production.

Future Research Directions and Limitations

While this study provides crucial insights into timing-specific LED responses, several areas warrant further investigation. The incomplete data for LED_22pm-2 am and LED_2-6 am treatments in leaf morphology parameters limit comprehensive comparison of night-time vs. early morning supplementation strategies. Future research should investigate the molecular mechanisms underlying these timing-dependent responses, particularly circadian clock gene expression and photosynthetic enzyme regulation patterns. The study's focus on morphological and yield parameters should be extended to include nutritional quality assessments, as recent literature suggests that LED timing may influence secondary metabolite production and antioxidant capacity. Based on the LED specifications (50 W/module), total installed power was ~150 W per treatment (3 modules), giving ~0.6 kWh/day (4-hour photoperiod) and ~21 kWh per treatment over the lighting period, or ~63 kWh across all three LED treatments. A full cost-benefit analysis was not performed, as this depends on fluctuating vegetable prices and additional capital costs beyond this study's scope.

However, prospects remain promising given improving LED efficiency and the growing use of solar power as a lower-cost, lower-emission alternative to fossil-fuel electricity in tropical greenhouse lighting. Additionally, longer-term evaluations could reveal whether the observed architectural changes translate into sustained productivity advantages under commercial growing conditions. The findings establish LED timing as a critical optimisation parameter for *Brassica juncea* production, with evening supplementation (18-22 pm) providing superior yield enhancement while revealing important morphological trade-offs that must be considered in precision agriculture applications.

CONCLUSION

This study demonstrates that LED photoperiod timing is critical for optimising *Brassica juncea* growth, with evening supplementation (18-22 pm) providing superior results. The LED_18-22 pm treatment achieved remarkable enhancements of 70.7% in plant height, 58.0% in total fresh weight, and 26.2% in leaf number compared to controls. However, this optimisation involved significant morphological trade-offs, including reduced leaf width (11.8%) and length (18.2%), with biomass allocation shifting dramatically from leaves (90.15% to 77.06%) to stems (1.92% to 17.33%). The temporal chlorophyll responses revealed stage-specific optimisation opportunities, with evening supplementation enhancing early development and early morning treatment improving mid-season chlorophyll content. These findings validate that 4-hour evening LED supplementation represents an energy-efficient strategy for maximising mustard green productivity while highlighting the importance of considering morphological consequences in commercial applications. The research establishes timing-specific LED protocols as essential tools for precision agriculture, demonstrating that when supplementation is applied is as critical as how it is applied for achieving optimal crop production outcomes. From a practical standpoint, the 4-hour evening LED supplementation (18-22 hours) required only a modest amount of electricity (~21 kWh per treatment over the growing period) while delivering a substantial yield gain (58%), indicating that this strategy is feasible for implementation in greenhouse or indoor production systems. In tropical regions with abundant solar radiation, pairing the LED system with solar power could further reduce operating costs and improve environmental sustainability, a direction that warrants dedicated evaluation in future studies.

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SAGO (*Metroxylon sagu*) Traditional Processing Practices and Challenges: An Exploration for Sustainability, Adaptability, Guidance and Optimisation (SAGO) Training

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ABSTRACT

Traditional sago processing in Aklan, Philippines has never been properly studied or improved. A sequential explanatory approach was used involving 35 sago processors. The study revealed that sago processors still use primitive manual methods, require a lot of hard labour, and harm the environment because of dumping untreated wastewater into local nature. This traditional knowledge leads to their hard conditions to survive. Based on the analysis of the study, sago processors have great technical skill, but they are held back by poor infrastructure, such as 71% lacking farm-to-market roads, and 88% relying on inefficient manual tools. The findings show that the industry survives purely on community teamwork and social bonds, like shared labour (86%), rather than actual support from the government or official institutions. Thus, the SAGO Training program was introduced in order to shift traditional practices into a sustainable business. This 12-month project is divided into three stages: curriculum design in line with RA 10611 and GMPs, hands-on implementation focused on optimising extraction and waste-to-value conversion, and impact assessment. The scalable model aims to save local industries while meeting modern safety and environmental standards. Its expected output includes professional certification for processors, reduced environmental pollutants through better waste management, and improved market access.

Keywords: Challenges, exploration, practices, processing, sago, sustainability, traditional, training

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INTRODUCTION

Sago palm is one of the Genus Metroxylon belonging to the Family Palmae. It has a high starch yield. One palm yields 150 -300 kg of starch, enough to feed a family of 4-5 members for a month (Sago Palm Society, n.d.). Thus, sago is considered by many scientists as the "starch crop of the 21st century" (Singhal et al., 2008). Aside from being nutritious, sago is great for the environment. According to Dewayani et al. (2024) and Purbaningsih et al. (2023), sago can survive weather changes and natural disasters, making it a reliable crop for securing food supplies locally and nationwide. Mohamed et al. (2024) also reiterated that in the Asia-Pacific region, this underused plant has the potential to become a highly valuable product for industries.

Despite SAGO having great industrial potential, its processing creates a lot of waste, such as bark, fibre residue (hampas), and wastewater (Awg-Adeni et al., 2010). According to the results of the investigations by Wee Oy et al. (2017) and Nagarajan & Suresh (2005), when this acidic wastewater is dumped without treatment, it degrades water quality, consumes the oxygen that aquatic animals need to breathe, and damages the tissues of fish and other aquatic life.

According to Teodosio (2013, as cited in Acuna, 2013), who conducted a survey pertaining to SAGO trees, large groups of these stands grow in 12[1] out of the 17 towns in Aklan Province, Philippines. The geographical distribution of sago stands in the Province of Aklan is presented in Figure 10. However, sago processing has never been officially recorded or improved; it still relies heavily on traditional local knowledge and manual labour. Although local laws, such as Republic Act No. 9003 and the Aklan Provincial Environment Code (Aklan Provincial Government, 2009), require proper waste management, actual processing methods remain inefficient.

Though sago has great economic and environmental potential, it lacks proper research and official documentation in the Philippines. In nearby countries like Malaysia and Indonesia, modern sago factories are being built, while the Philippines lacks hard data on how old manual methods, like slow extraction and inefficient tools, cause people to dump waste in ways that harm the environment. At present, there is no proven plan to help sago processors combine these ancient traditions with modern food safety and environmental standards.

This investigation is based on Socio-Technical Systems (STS) Theory. According to Trist and Bamforth (1951), this theory states that how well a business or group works depends on how its Social Part (the people, traditional knowledge, and customs) interacts with its Technical Part (the tools, processing methods, and waste management).

[1]Please see Figure 1 (Geographical map of sago strands in the province of Aklan) above the Subheading: Participants/Informants and sample size of the study

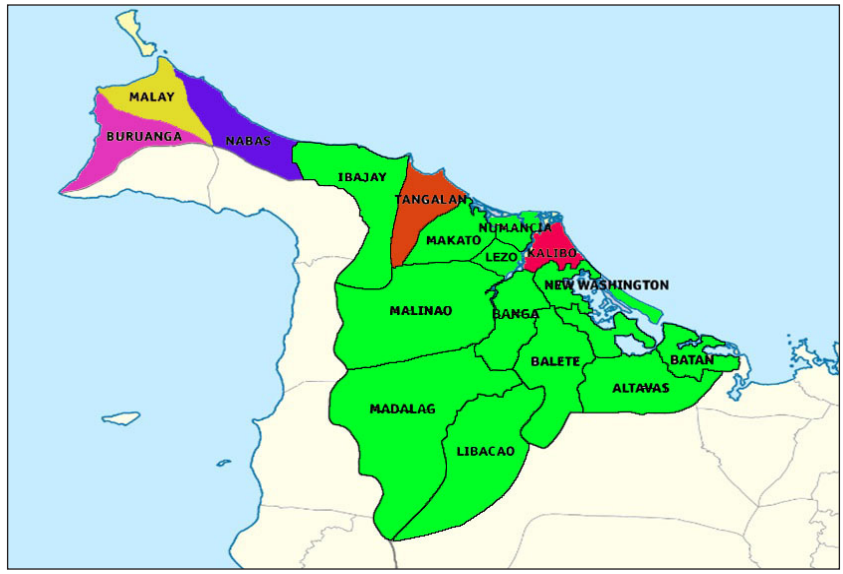


Figure 1. Geographical map of sago strands in the province of Aklan
Note. Green colour on the map is the identified municipalities in the province of Aklan where sizeable patches of sago stands can be found.

Independent variables include Traditional Processing Practices, which encompass Selection, Rituals, and Extraction. These variables interact with intervening factors, such as Internal and External Challenges, which consist of labour intensity, lack of transportation infrastructure, and technical gaps. The dependent variable in this context is the Sustainability of the sago Industry. To address these issues, the SAGO Training Program, focusing on Sustainability, Adaptability, Guidance, and Optimisation, serves as a proposed intervention to align social traditions with technical efficiency.

Statement of the Problem

The main goal of this study is to closely examine the environmental impacts and day-to-day risks of traditional sago (*Metroxylon sagu*) processing. Specifically, it aims to answer the following questions:

1. What are the environmental and socio-economic impacts of traditional sago processing practices in the province of Aklan, Philippines?
2. What are the waste-management challenges and the ecological risks associated with current byproduct disposal?
3. What are the critical governance gaps and resource needs that hinder the efficiency and formalisation of the industry?
4. What training program can be designed to enhance the sago industry based on the results of the study?

MATERIALS AND METHODS

This study used a mixed-methods research design, specifically a sequential explanatory approach. According to Creswell and Plano Clark (2018), it is conducted to improve and explain the first statistics in which this method gathers and examines quantitative data and then a qualitative inquiry. This was chosen because neither approach alone could adequately capture the technical inefficiencies of sago processing as well as the lived experiences and the "why" behind traditional practices. By integrating both datasets, triangulation is achieved, whereby the researcher can check for statistical trends with detailed participant views (Creswell et al., 2003).

Aliaga and Gunderson (2002) reaffirm that the quantitative phase was to quantify technical challenges and baseline socioeconomic data of sago processors. There were 39-target population of sago starch processors in 12 selected municipalities in the Province of Aklan. The researcher used stratified random sampling to ensure representation from different geographic locations. The sample size was determined by using Slovin's Formula, which was 35 respondents. The data were collected by means of a researcher-made structured survey questionnaire that gave statistical data on processing volume, waste output and economic indicators.

After analysing the quantitative data, the researcher focused on the workers' personal stories and how they handle daily hardships (Creswell, 2003). For this part, 12 key experts were handpicked from the original survey group based on their deep knowledge and long experience in the sago industry. The researcher gathered their insights through group discussions and detailed, one-on-one interviews. With this, it gives the researcher a clear picture of why processors use traditional waste disposal methods and where local government support is missing.

Data integration occurred at the interpretation stage, where qualitative narratives were used to "dig into" the quantitative results. Creswell et al. (2003) point out that this triangulation serves to contrast data points and validate whether the statistical trends aligned with the participants' actual experiences in the field.

Since the researcher acknowledges their role as a neutral tool, the researcher practised reflexivity by keeping a research journal to monitor preconceptions and ensuring that the qualitative "voice" of the traditional processors was not overshadowed by statistical goals, that is, to maintain objectivity. Through this awareness, it helped ensure that the proposed SAGO Training remains culturally sensitive and is anchored on the actual needs of the Aklan processors, not a top-down imposition of technology.

Participants/Informants and Sample Size of the Study

Purposive sampling was used by the researcher in identifying the primary participants. The researcher coordinated with the Provincial Agriculture Office for the list of municipal

agriculture officers and asked for their assistance to identify sago starch processors in their respective communities. A total of 35 sago starch processors were identified as respondents of the study. They answered the researcher-made survey questionnaire and were allowed to narrate their lived experiences pertaining to sago starch processing. Due to COVID-19 restrictions and health protocols, the researcher used snowball sampling in order to have additional/replacement participants during the conduct of the survey and interview.

Demographic Profile of the Respondents

The demographic characteristics of the respondents are presented in Figures 2 to 10. These figures provide a comprehensive overview of the respondents' profiles in terms of gender, age, educational attainment, marital status, primary occupation, monthly household income, primary sources of income, land ownership status, and land area distribution. Understanding these characteristics provides essential context for interpreting the findings of the study.

Figure 2 presents the distribution of respondents according to gender, while Figure 3 illustrates the age distribution of the respondents.

Figure 4 illustrates the educational attainment of respondents, while Figure 5 displays their marital status.

Figures 6 and 7 illustrate the primary occupation and monthly household income of the respondents.

Figure 8 illustrates the primary sources of income for respondents, while Figure 9 displays their land ownership status. Additionally, Figure 10 below presents the area of sago palm land owned by the respondents.

As cited by Babbie (1995) and Crabtree & Miller (1992), Snowballing is a method of expanding the sample by asking one informant or participant to recommend others for interviewing. The researcher gave the local agriculture officers the authority to choose the farmers/sago processors that met predetermined criteria and recommend them as appropriate additional participants, especially in dealing with the focus group discussions/in-depth interviews. Local residents and some of the provincial/municipal agriculture officers served as key informants during the interviews.

Research Instrument

In order to address the study's analytical objectives and to capture the technical, environmental, and socio-economic dimensions of the sago industry in Aklan, the research instrument was made comprehensively and divided into four specialised sections. The first section focuses on the General Information of the respondents, consisting of nine items that detail their socio-economic profile, including sex, age, income, and land ownership. It gives basic information about the processors and looks at the resources they can access.

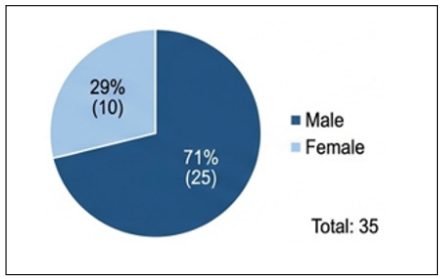


Figure 2. Distribution of respondents according to gender

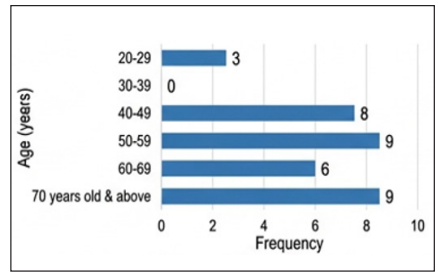


Figure 3. Distribution of respondents according to age

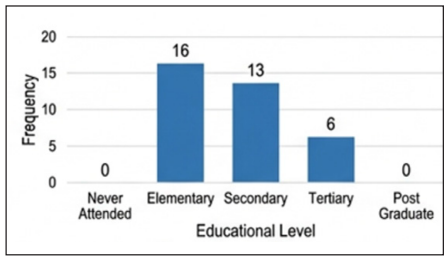


Figure 4. Distribution of respondents according to educational attainment

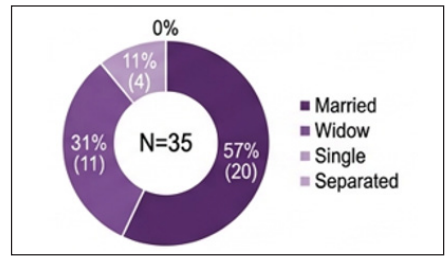


Figure 5. Distribution of respondents according to marital status

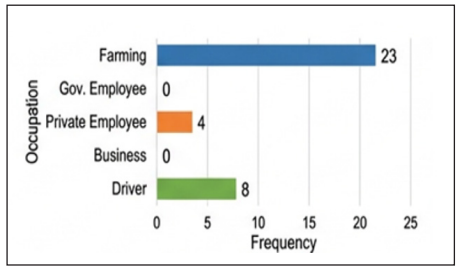


Figure 6. Distribution of respondents according to primary occupation

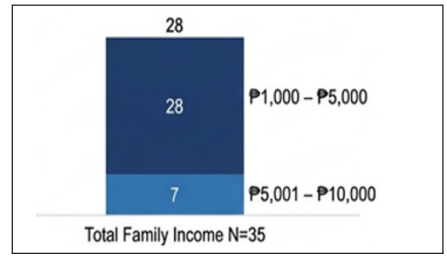


Figure 7. Distribution of respondents according to monthly household income
Note. Family monthly income - P 60.69 Philippine Peso = $ 1 US Dollar (as of March 2026)

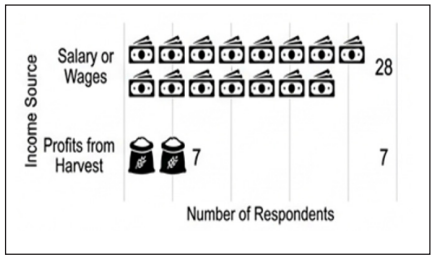


Figure 8. Distribution of respondents according to primary sources of income



Figure 9. Distribution of respondents according to land ownership status

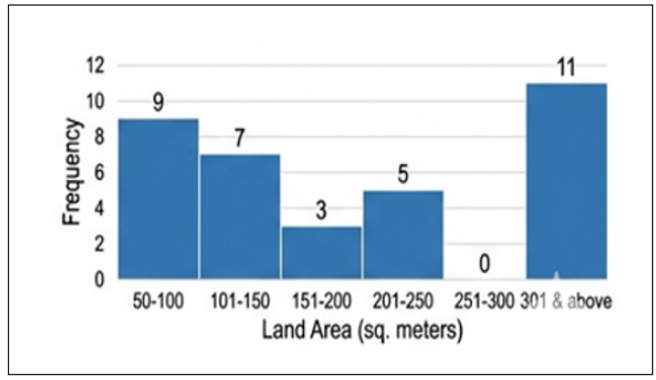


Figure 10. Distribution of respondents according to sago palm land area

The second section is the sago processing audit. It lists 12 main items to track details about labour, tools, time, and how much sago they make. These items helped find where work slows down and how resources are used in both the dry and wet processing methods used in the area. This part of the instrument is practical, almost mechanical, but it reveals where efficiency is lost.

Next comes the waste disposal section. Seven detailed questions explore environmental risks by examining the types of waste produced: bark, hampas (fibre residue), wastewater and recording how much is discarded and where it ends up.

Finally, the fourth section looks at challenges using 10 rating-scale questions and three open-ended questions. It measures major problems, like a lack of government support or poor equipment. It will help design the SAGO training program.

The questionnaire was developed through a three-stage process. Items were derived from the operational definitions of "Sago Starch Processing" and "Waste Disposal Practices". Questions were arranged chronologically to follow the actual flow of production from cutting and debarking to packaging and marketing, ensuring no stage was omitted. Segment here specifically asks about interest in technical knowledge and government-led training, directly feeding into the SAGO Training output.

For the reliability of the survey, experts in Sago processing first reviewed the draft to verify if the questions matched the actual steps and sustainability goals of the study. A pilot test followed, involving a small group of processors from a town outside the main sample. As in the case of the "Challenges" part, Cronbach's Alpha was used to confirm that the questions consistently measured how difficult the tasks felt. Some participants repeated the survey after a short time to confirm their answers stayed stable. Finally, open-ended questions, such as asking about interest in training, were included to add depth and verify the checkbox responses, helping keep the data clean and meaningful.

Data Gathering and Analysis Procedure

Twelve (12) municipalities in the province of Aklan participated in the study. The researcher used a triangulation approach to identify gaps in waste disposal practices and industry challenges. To guarantee a comprehensive dataset, quantitative surveys were paired with qualitative interviews.

Permission was formally secured from both the Provincial and Municipal Agriculture Offices. Self-administered questionnaires and structured, in-depth interviews were used to gather data. While a large number of processors were reached by the primary survey, key informants for qualitative investigations were found through purposive sampling and snowballing. The data collection period was done on June 28 to August 7, 2021.

In adherence with COVID-19 health protocols, social distancing was observed, written consent was required, and respondents were encouraged to rely on gestures and body language to minimise risk during face-to-face interactions. Participants were also briefed on data management and confidentiality and were informed that the intention of the investigation is for research and publication.

A mixed-methods approach was used for analysis. Statistical rigour was combined with the depth of phenomenology. The questionnaire data were totalled and tabulated utilising Microsoft Excel. Descriptive statistics such as mean, rank, frequency, and percentage distributions were applied to disposal practices and industry challenges. Whereas to identify more general patterns and connections in the dataset, inferential techniques were used.

As for the qualitative phase, capturing the "lived experiences" and "adaptive resilience" of sago processors was the goal. It started with bracketing, putting aside the researcher's preconceptions to ensure objectivity, in accordance with Moustakas's (1994) framework. Then, the researcher used a methodical six-step procedure: 1) Familiarisation: Verifying the accuracy of the data and finding any gaps; 2) Coding: Generating initial codes using highlighters on hard-copy transcripts; 3) Thematic Discovery: Identifying significant patterns related to the research questions; 4) Reviewing Themes: Consolidating relevant data points under each theme; 5) Finalisation: Naming and refining themes based on their essence; and 6) Documentation: Relating the themes back to literature and research objectives.

For a holistic view, the researcher developed: textural descriptions by detailing what processors experienced (e.g., difficult extraction tasks) and structural descriptions by detailing how they experienced it (e.g., the impact of poor tools and lack of market access). Figure 11 below shows the flow chart of the data gathering procedure.

All recorded interviews were transcribed on the same day they were conducted to prevent ambiguity or loss of context and to maintain internal validity.

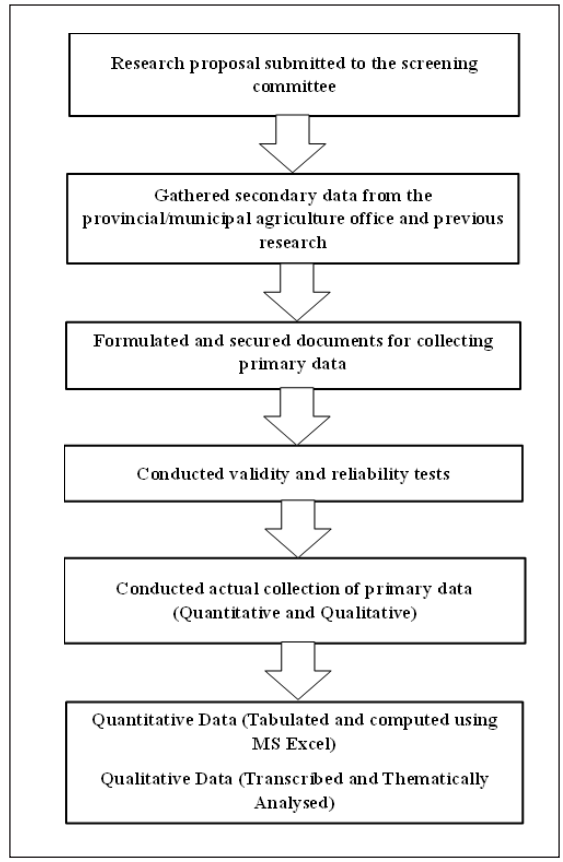


Figure 11. Flow chart of data gathering procedure

The synthesis of these methods was designed to optimise SAGO Training. Through understanding the "human" context behind traditional practices, the researcher ensured that the resulting training programs were both technically sound and culturally adaptable for Aklanon processors.

RESULTS AND DISCUSSIONS

Traditional Processing Practices in the SAGO Starch Production

Based on the interview conducted by the researcher, the sago processors in Aklan enumerated their traditional processing practices in sago starch production by: A) Identifying mature sago palm through different physical inspections; B) Rituals in cutting the sago trunk; C) Using traditional/manual tools/strategies during sago starch processing; and D) Two traditional methods of extracting sago starch. Data gathered pertaining to traditional practices in the sago starch production are presented in Figure 12.

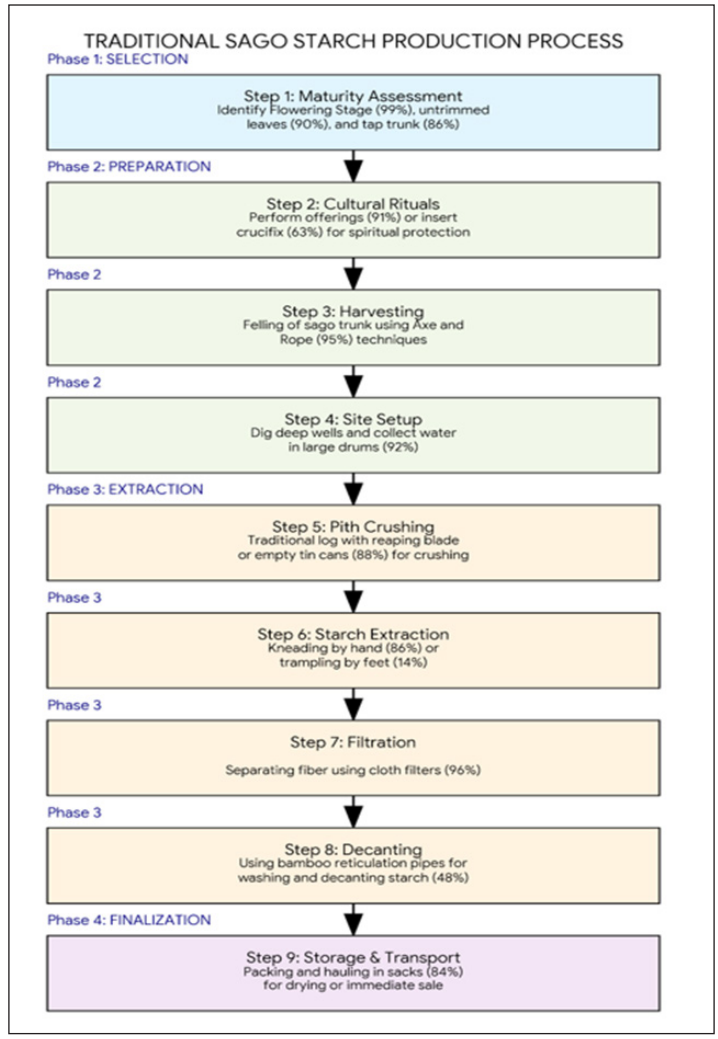


Figure 12. Traditional processing practices in sago starch production

Sago processors did different physical inspections in identifying the most desirable trunks (in terms of age and starch content) through the flowering stage (99%), conditions of leaves (trimmed or untrimmed) (90%), tapping the trunk (86%), water content after debarking (80%), and appearance of roots (43%). Wan (2016) attested that the best harvesting time for sago palm is the beginning of the "*inflorescence stage*" before the "*fruit ripening stage*".

Sago processor also prepare several rituals before they cut the sago trunk such as putting a small bundle of freshly squeezed sago starch as an offering (91%); making a crucifix made from small sago palm dry leaves and inserting it in its trunk (63%); rubbing

of ginger on the bit/wedge of the axe (40%); put ginger in the mouth those who will cut the sago trunks (40%) and place two tikog leaves near the sago trunk and tie these tikog leaves (14%). This traditional sago processing in Aklan is governed by spiritual and cultural safeguards intended to ensure an abundant harvest and appease supernatural entities, a practice mirroring the Toradja of Indonesia, who offer sago to deter spirits from processing sites (Adriani & Kruyt, 1951). These customs include strict gender-based taboos, such as the exclusion of pregnant or menstruating women to prevent the perceived failure of starch to settle in water, a belief reflecting broader regional traditions in Papua New Guinea that associate menstruation with impurity and the potential disruption of communal tasks (Mohamed et al., 2018).

Sago processors enumerated their traditional/manual tools/strategies during sago starch processing. They filter sago starch using cloth (96%), employ axes and ropes to cut the trunks of sago palms (95%), and dig deep wells near the processing site to collect large quantities of water (92%). For crushing the sago pith, they attach reaping blades or empty sardine cans to bamboo or wooden logs (88%) and use sacks for packing, hauling, and storing the starch (84%). Additionally, they create reticulation pipes from split bamboo to collect water for washing and decanting starch (48%). These processors prefer manual felling over chainsaws to avoid damage from resin, followed by a critical pounding or rasping stage that breaks down the pith's cellular structure to release starch (Wan, 2016). In regions like Papua New Guinea, metal-reinforced wooden pounders are used (Toyoda, 2018), and the final yield is influenced by the “fineness” of the rasping process to ensure efficient starch washout during rinsing (Darma, 2018). Following extraction, communities employ various storage methods, such as dry, leaf-wrapped bundles or long-term wet storage in canoes or water-filled containers (Greenhill, 2006; Pue, 2013).

Sago starch extraction can be categorised into two types, the traditional and modern. However, principles of sago extraction for both methods are similar, while the differences are only in the scale of operation (Kamal et al., 2007 & Karim et al., 2008) and the processing tools that are used (Greenhill, 2006). According to sago processors, they practised the traditional way of extracting the sago starch by means of kneading by hand (86%) and trampling by feet (14%). They further explained that trampling by feet extracts more starch than kneading by hand and is not tedious.

Sophisticated fusion of indigenous knowledge, spiritual heritage, and economic practical results is reflected in Aklan's traditional sago industry. Sago processors use ecological knowledge to increase starch yields by scheduling harvests during the inflorescence stage. With its cultural customs and taboos that regard the sago palm as a sacred resource, this environmental conservation gives the community a sense of social solidarity and spiritual grounding.

Economically wise, sago processors prefer using simple, low-cost tools instead of expensive machines. Oftentimes, they make improvised equipment, like cloth filters, resin-resistant axes, and scraping devices from household waste. By doing this, it keeps costs low while still producing good results. sago processors also knead the starch by hand or use trampling for higher yield. These methods show a strong production system that balances effort and output.

Sago Waste Disposal Practices and Utilisation of Sago By-Products

Sago waste disposal practices refer to the repeated habitual or regular manner or procedure of an Aklanon in throwing away or getting rid of the substance or material such as bark, hampas and wastewater after its primary use in sago processing.

Data gathered pertaining to sago waste disposal practices and utilisation are presented in Figure 13, as it features the manner of eliminating and utilising these three sago wastes generated after the production of sago starch, namely sago bark, SAGO pith (hampas) and sago wastewater.

The data showed that 43% of the sago processors threw the sago bark in the river or left it on the site; 31% of them did the same for its sago pith (hampas), while its sago wastewater was 100% thrown in the river or left on the site. However, 46% of sago processors used sago bark as fuel, and 11% utilised it as wall for their houses. Meanwhile, there were 49% of sago processors who used sago pith (hampas) as feed for their backyard animals, and 20% of them utilised it as compost for their vegetable garden.

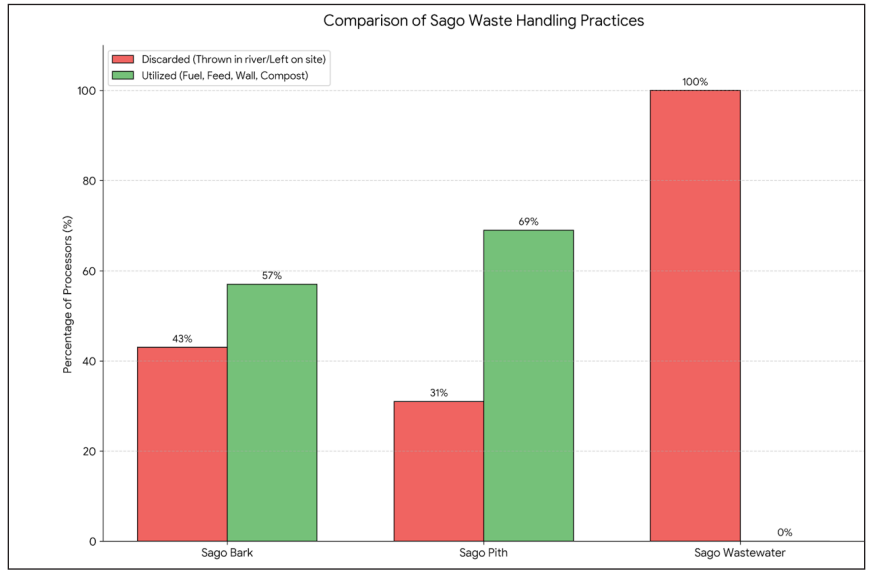


Figure 13. Sago waste disposal practices and utilisation of its by-products

In the research conducted by Murod et al. (2018), sago bark is the main solid waste from sago processing and has traditionally been used for things like machine bases, load supports, or as extra fuel. Nishimura (2015) cited that in large operations, such as in Malaysia, processing about 600 logs in one day can produce around 15.6 tons of bark, along with large amounts of wastewater and fibre waste. However, Amin et al. (2019) and Murod et al. (2018) contend that though sago bark is abundant, it is not fully used, it showed that only about 15% of shredded bark is burned as fuel to dry flour in sago mills, while most of it is simply burned to prevent buildup.

Sago pith, also called *hampas*, is a starchy by-product produced in large amounts during starch extraction. According to the study of Awg-Adeni et al. (2010), in places like Sarawak, Malaysia, daily output of sago pith can reach 50-100 tons. Because it still contains a lot of starch, hampas is often used as a cheap feed for pigs and ruminants, with nutritional value similar to sweet potato waste (Singhal et al., 2008). Aside from being used as animal feed, sago processors also utilise it as compost for vegetable gardens, and leave piles of hampas on-site, which encourages the growth of SAGO worms that thrive in the decaying material.

During the extraction of sago starch, it requires a water-intensive process, with liquid waste comprising 94-97% of the total output. This results in the daily discharge of 10-22 tons of wastewater per factory, characterized by an exceptionally high carbon-to-nitrogen ratio (105:0.12), which leads to the severe contamination of local river systems, evidence provided by Phang et al. (2000). Moreover, current practices reflect an "enormous" waste of water, as SAGO processors continue rinsing until the runoff runs clear, discarding the effluent without treatment.

Awg-Adeni et al. (2010) and Amin et al. (2019) posit that, nonetheless, research into biotechnological advancements suggests that these residues can be transformed into economic assets through value-added product development. Moreover, implementing established waste-utilisation models, such as those developed by Wan Samiati et al. (2010), Shaari (2014), and Umar et al. (2018), offers a pathway to mitigate environmental impact while providing sago processors with sustainable, alternative income streams through proper technical training.

Traditional disposal methods, like dumping waste in rivers or leaving it on-site, lead to conflict with environmental sustainability and are problems faced by Aklanons. These practices are cheap for sago processors but cause issues such as water pollution, eutrophication, and harm to local fisheries. There is a better option, where sago waste can be turned into useful products like eco-friendly building materials, compost, or renewable energy from biogas and bio-ethanol. So as to make this shift possible, training and workshops are needed to help local processors learn how to turn waste into economic opportunities.

Challenges and Issues of Sago Processors in Sago Starch Processing

Challenges and issues feature the difficult tasks or problems encountered by the respondents during sago processing from cutting to marketing. Figure 14 condenses the challenges and issues of sago processors in relation to sago starch processing.

The researcher summarised the challenges and issues extracted from the interviews, identifying several internal and external factors. Internal challenges included time consumption, rated at 100%, followed by the need for substantial manpower at 86%, limited water sources at 57%, poor tools and equipment at 46%, and limited technical knowledge at 26%. External challenges featured inadequate farm-to-market roads, rated at 71%, a lack of government support at 37%, an absence of a clear market at 26%, and insufficient waste management systems, along with a lack of political will and motivation, both rated at 14%.

Traditional sago starch processing faces significant internal operational challenges, particularly due to its intensive time and labour requirements. According to Pue et al. (2018), the process can take more than a full day, depending on the proximity to water and the available workforce. Loreto et al. (2006) noted that completing the extraction of a single sago log can take between 5 to 6 days and requires a team of 10 to 12 workers, which underscores the tedious nature of traditional methods.

Water scarcity poses a major challenge in the Aklan sago industry, compounded by limitations in resources and technology. Research studies by Nishimura (2015), Karim et al. (2008), and Singhal et al. (2008) indicate that transitioning from traditional manual horizontal methods to vertical mechanical water-flow systems can enhance efficiency. This shift has the potential to produce starch that is cleaner, of higher quality, and more hygienic.

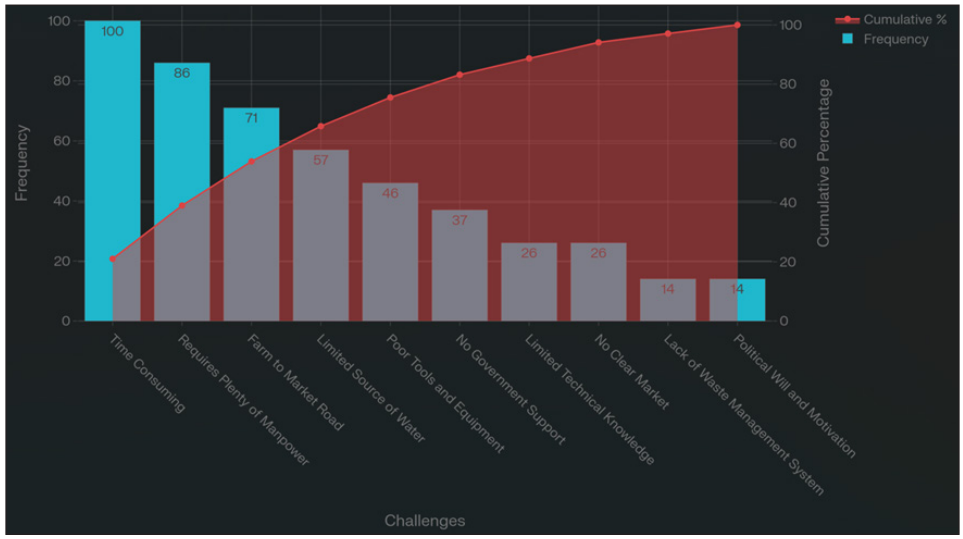


Figure 14. Challenges and issues of sago processors in sago starch processing

Sago processors are forced to spend excessive time on kneading and washing due to the use of substandard and traditional tools. This reliance on outdated methods is often attributed to a technical knowledge gap, as processing techniques are typically inherited from previous generations rather than informed by modern optimisation (Loreto et al., 2006). Furthermore, the sago industry in Aklan has significant external pressures that threaten its sustainability, primarily because of logistical isolation and institutional neglect. Because sago palms thrive in swampy, interior forest regions, the lack of adequate farm-to-market roads remains a critical barrier to efficient transport. Saediman and Taridala (2006) note that this is compounded by a lack of government support and the absence of a clear market, forcing sago processors to rely on limited buyer information, which often prevents them from making profitable decisions.

Systemic issues further marginalise the industry despite demand spikes from Boracay's tourism sector. These include market volatility and inconsistent orders, which often result in mature palms being left to rot in the field. In addition, social and generational decline, where a growing "generational gap" exists, as modern youth show little interest in sago farming, leading sago processors to harvest primarily for subsistence or small-scale local sales. Lastly, governance & environmental gaps, in which lack of political will among local officials has resulted in sago concerns being excluded from community priority projects, leaving the industry without essential waste management systems or modern infrastructure.

Overall, the sago industry in Aklan is struggling both inside and outside the processing sites. Internally, workers rely on slow traditional methods that they find difficult to manage, made worse by limited water supply and outdated tools. Switching to mechanical washing could improve speed and cleanliness. Externally, the industry suffers from weak infrastructure, unclear markets, and little political support, forcing processors to depend on informal buyers. This neglect, combined with declining interest from younger generations, threatens the future of the industry. Without modernisation and better market access, sago processing will likely remain a small-scale, subsistence activity.

Sago Processors Coping Mechanisms in Sago Starch Processing

Processors of sago in Aklan have developed various strategies to cope with the challenges of starch production. Some of these strategies are practical, while others are more adaptive, showcasing how communities manage to sustain themselves despite limited support.

Figure 15 illustrates these coping mechanisms. For internal challenges, time management stands out, with 100% of processors relying on it. Next, division of labour was rated at 86%, highlighting the importance of teamwork. Resourcefulness received a score of 57%, innovation 46%, and seeking advice from experts only 26%. For external challenges, 71% of respondents noted the use of initiatives, while adaptability was mentioned by 37%.

Resilience and responsibility, along with optimism, were each indicated by 14%. These numbers tell a story: processors primarily rely on collaboration and initiative. In contrast, optimism and resilience, while present, are less commonly utilised.

Aklanon sago processors utilise a variety of coping mechanisms, characterised by resourcefulness, social collaboration, and local innovation, to navigate the significant challenges of starch production. Facing the time-consuming nature of sago processing, workers have adapted their schedules by cutting trunks into smaller logs, allowing them to manage their workload without having to finish an entire tree in one day. They also lessen labour demands through teamwork and by scheduling production during less busy periods in the rice farming cycle. To address the issue of limited water access, they dig deep wells near processing sites and use large drums to collect water for washing. Additionally, when tools are inadequate, sago processors create their own equipment, much like practices in Papua New Guinea, where wooden pounders are reinforced with metal tips or nails to enhance efficiency (Darma, 2018; Toyoda, 2018).

However, there are knowledge gaps among sago processors that need to be addressed. Through peer learning and community consultations, there is an identified urgency for formal technical training, particularly in processing and waste management, in order to modernise the sago industry (Rasyid et al., 2020).

Sago processors in Aklan face numerous challenges from both their operational systems and the surrounding environment. Despite these obstacles, they manage to continue their work by relying on local solutions, demonstrating strong resilience, and maintaining personal optimism to sustain their livelihoods.

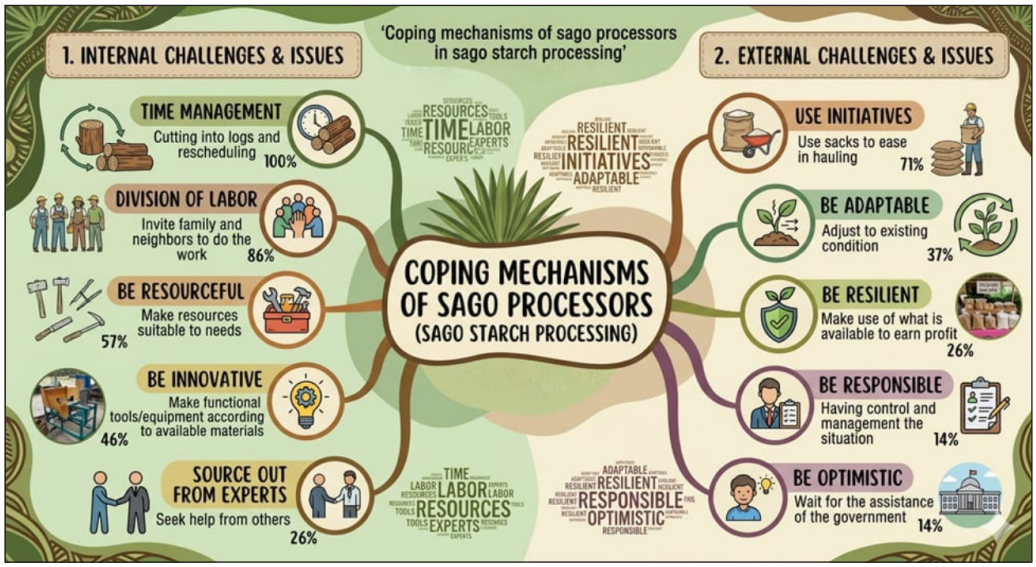


Figure 15. Coping mechanisms of sago processors in sago starch processing

As Jong (2018) noted, common challenges for sago industries include geographical remoteness and poor farm-to-market roads. In response to these issues, sago processors employ resourceful methods for storage and transportation. They safely manage and transport their starch using woven natural fibre bags, plastic containers, or ceramic pots (Greenhill, 2006).

In the absence of government support and clear markets, sago processors have adopted an adaptive coping strategy, perceiving these stressors as conditions to be managed rather than insurmountable threats (Southwick et al., 2005). However, long-term sustainability requires a shift toward "greener technology," such as the Argao dry-processing method, which minimises waste (Flores, 2016), alongside formal training for farmers in waste management (Rasyid et al., 2020).

Despite a lack of formal waste management systems and poor regulatory enforcement on effluent disposal (Awg-Adeni et al., 2010), processors take individual responsibility by piling waste in specific areas to mitigate immediate environmental damage. Sago processors maintain a mindful, optimistic outlook to navigate the lack of political motivation in their communities (McKee et al., 2008). Nevertheless, research concludes that true industry sustainability is impossible without government-led incentives, capacity building, and improved financial institutional support (Girsang, 2018).

Sago processors in Aklan keep their livelihoods going through teamwork, creativity, and resilience, adapting to the hard work of starch extraction with careful scheduling and improvised tools. They deal with poor infrastructure and little government support by taking responsibility for environmental impacts themselves. Still, long-term sustainability will depend on proper technical training, the use of greener technologies, and government incentives. Many processors believe the sago palm could help drive economic recovery if supported with modern machinery and better ways to use waste.

Output of this Study for Sago Industry Enhancement

This study presents an overview of traditional sago processing, waste disposal, and the challenges faced in Aklan, Philippines. The primary aim is to strengthen the sago industry and enhance waste management by proposing training programs for sago processors and their families. The training will emphasise sustainability, adaptability, guidance, and efficiency, while also addressing the use of waste, mental health concerns, and providing additional livelihood opportunities. Drawing inspiration from initiatives in other countries, the study seeks to demonstrate how sago waste can be transformed into valuable products that benefit both the community and the environment.

Furthermore, the study highlights that while sago processors in Aklan are resilient in coping with challenges, their efforts tend to focus on mere survival. Poor tools and limited market access keep them trapped in a cycle of poverty. To transform sago from a small,

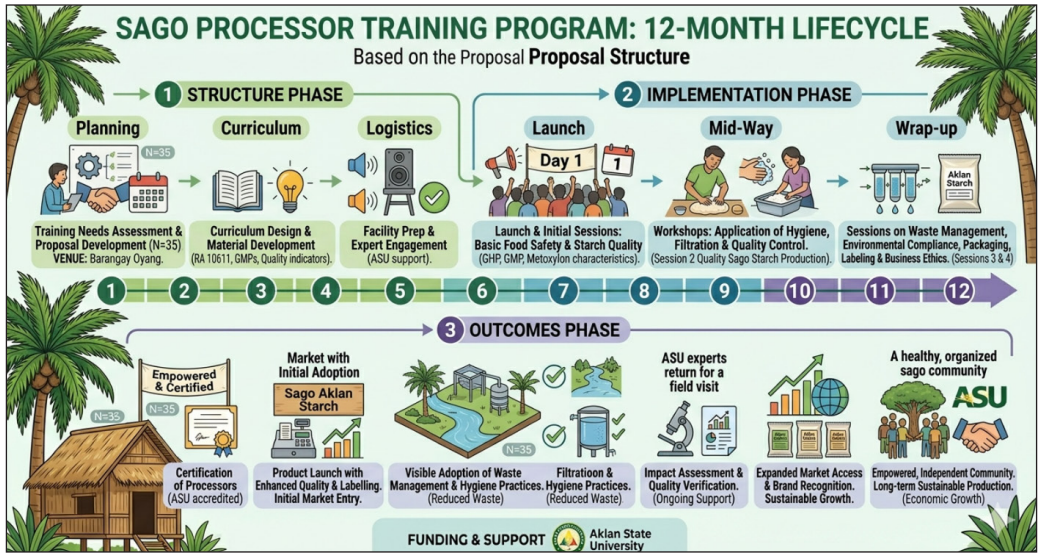


Figure 16. Sustainability, Adaptability, Guidance and Optimisation (SAGO) Training Proposal

struggling endeavour into a more robust economic resource, support must extend beyond individual resilience and involve institutional backing. Providing modern machinery and technical training would not only save time and enhance efficiency but also help rebrand the industry as a modern and sustainable livelihood for future generations. Figure 16 shows the outline of the SAGO training proposal.

CONCLUSION

This paper highlights the cultural and economic importance of sago processing in Aklan, Philippines. It recognises the various efforts and experiences of local sago processors. The proposed SAGO Training Program aims to enhance the sustainability of the industry, convert waste into valuable products, and protect local ecosystems.

Based on the discussions, key recommendations include improving infrastructure with support from local government, enforcing waste management regulations for sago mills, establishing a dedicated "SAGO Desk" to assist processors, and promoting modern technology and product diversification to raise industry standards. The S.A.G.O. model could potentially be applied in other regions as well, but additional research on biotechnology and the involvement of youth is necessary to ensure long-term growth and revitalisation of the sector.

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CONFLICTS OF INTEREST

The authors declare no competing interests.

DISCLAIMER

During the preparation of this work, the authors used ChatGPT (OpenAI), Google Gemini and Microsoft Copilot in order to assist with drafting and language editing, structural revision of the manuscript in response to peer-review feedback, preparation of supporting figures and verification of references against publicly available regulatory and bibliographic sources. After using these tools, the authors reviewed and edited the content as needed, independently verified all factual claims, data values, and citations against primary sources and take full responsibility for the content of the published article.

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Growth and Reproductive Development of Asian Seabass, *Lates calcarifer* in Small-Scale Aquaculture Farms in Malaysia

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ABSTRACT

Understanding the relationship between production management and protandrous hermaphroditism in farmed Asian seabass (*Lates calcarifer* Bloch 1790) is vital for optimising aquaculture efficiency. This study evaluated the growth performance and gonadal development of Asian seabass (n = 112) across six open and semi-closed small-scale farms in Peninsular Malaysia. With the exception of one farm population that displayed negative allometric growth ($b < 3$), fish growth was isometric ($b = 3$) across the remaining groups. Non-parametric Mann-Whitney U tests revealed that semi-closed systems achieved significantly higher performance metrics than open configurations, where the relative condition factor (K) was significantly elevated (median 1.30 vs. 1.18; W = 172, $p < 0.001$), as was the Specific Growth Rate (SGR, median 1.01 vs. 0.99 % day^{-1}; W = 279.5, $p = 0.046$). Multi-model information-theoretic selection (AIC_c) confirmed that farming practice models that combine enclosure method and water-source environment offered the highest predictive support (model weight, W_t = 76%), outperforming models based on broad system (semi-closed vs. open) configurations alone. Histologically, ordinal logistic regression models revealed that water salinity environmentally drives sex reversal in the farmed fish. Holding total length constant, fish reared in low-salinity environments (≤ 25 ppt) exhibited 9.1 times higher odds of occupying advanced gonadal stages compared to high-salinity cohorts (> 25 ppt), significantly compressing both male-peak and female-transition size thresholds. Ultimately, these findings demonstrate that while semi-closed infrastructure accelerates daily growth velocity, small-scale farmers must prioritise matching specific culture methods to environmental salinity profiles to manage growth and sex reversal timelines effectively.

Keywords: Asian seabass, precocious maturation, protandrous hermaphroditism, salinity modulation, small-scale aquaculture

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INTRODUCTION

Optimising growth performance is a primary objective in aquaculture production. This is because faster growth shortens production cycles, which in turn increases biomass yield (Small et al., 2016). However, growth and reproduction are physiological processes that compete for energy resources. For example, increased allocation of energy towards reproductive development may reduce somatic growth, and rapid growth can also alter the timing of sexual maturation (Bogevik et al., 2014). In aquaculture, early or precocious sexual maturation is generally undesirable because energy is diverted from muscle deposition to gamete production. This would ultimately reduce growth efficiency and harvest quality (Felip et al., 2008; Taranger et al., 2010). Such processes are regulated through the hypothalamic-pituitary-gonadal (HPG) axis. In the HPG, nutritional status, body size, and growth rate influence endocrine signalling and the expression of genes involved in reproduction (Ding et al., 2026). Therefore, understanding how farming conditions influence these physiological processes is important for improving management practices in both grow-out and broodstock production.

The relationships between farming practices and the respective physiological responses are important in protandrous hermaphroditic species such as the Asian seabass (*Lates calcarifer* Bloch, 1790). As a catadromous species, Asian seabass generally matures first as a male in lower-salinity riverine habitats. The fish would then migrate to higher-salinity marine waters to undergo sex reversal to become functional females (Rossi et al., 2018). This reproductive plasticity enables the Asian seabass to be cultured across a wide range of salinities during the grow-out phase (Hassan et al., 2024; Yusof et al., 2024). Hatcheries can produce Asian seabass larvae at salinities of 33 ppt or higher (Young & AlMoutiri, 2022). However, the fish achieve optimal growth and feed conversion in brackish water between 5 and 15 ppt (Wijayanto et al., 2021; Yusof et al., 2024). Such environmental flexibility has contributed to the ubiquitous rearing practices of Asian seabass in both marine and inland aquaculture.

Small-scale aquaculture enterprises dominate Asian seabass farming in Malaysia and across Southeast Asia. Typically, these farms operate with limited infrastructure, scarce resources, and minimal environmental control (Abdullah et al., 2018). Most rely on either open or semi-closed production systems. Open systems, which mainly use floating cages in coastal or riverine waters, depend entirely on natural water exchange. While they require very little capital upfront, they leave fish vulnerable to wild environmental shifts, disease outbreaks, and sudden water quality drops (Venkatachalam et al., 2018). Alternatively, semi-closed systems like inland earthen ponds and flow-through tanks offer better control via active water treatment (Turlybek et al., 2025). However, the trade-off is financial, where running these systems demands higher operating costs and strict management to stop waste from accumulating (Rud et al., 2017). Because most smallholders cannot afford

these rigorous feeding programs or salinity controls, production performance varies wildly from farm to farm (Mohd Nor et al., 2019).

In Malaysia, Asian seabass remains a premier cultured brackishwater and marine finfish (Department of Fisheries Malaysia, 2024). Brackish water ponds drove the bulk of production volume in 2024, yielding 52,677.95 tonnes. This translated to a wholesale value of RM722.8 million and a retail value of RM837.5 million (Exchange rate: RM1 = USD0.22, December 2024). By contrast, floating cages produced a fraction of that volume at just 8,183.61 tonnes, yet their wholesale value per tonne was roughly 62% higher than pond-raised fish. This stark valuation gap points to a clear market premium for cage-cultured seabass, likely driven by superior fish quality and distinct production methods. The structural advantage of cage culture, namely constant water exchange, has long been linked to better growth rates and sharper feed conversion efficiency (Anil et al., 2010).

While small-scale Asian seabass farming is economically vital, we still know very little about how commercial culture systems influence growth and reproduction. Most data comes from controlled laboratory or hatchery trials focusing tightly on artificial spawning, sex differentiation, and endocrine regulation (Athauda et al., 2012; Donaldson, 1996; Harvey et al., 1985; Heidari et al., 2025; Ravi et al., 2014). How these biological processes play out in the unpredictable environments of commercial open and semi-closed farms remains a blind spot. Intensive culture pushes rapid somatic growth, which can accelerate male differentiation and trigger earlier sex reversal as compared to wild populations (Terence et al., 2021). This shifts the biological timeline. In practical terms, these shifts disrupt harvest size uniformity during grow-out and throw off the male-to-female ratio needed for sustainable broodstock management. Therefore, this study aimed to evaluate the growth performance and reproductive development of Asian seabass cultured in smallholder farms across Peninsular Malaysia. Mapping these relationships on active commercial farms is critical to upgrading production efficiency and securing long-term broodstock survival.

MATERIALS AND METHODS

Farm Practice Characterisation

Samples were collected from March to October 2023 at six small-scale commercial Asian seabass farms located in Peninsular Malaysia. All farms were located along the coastal regions bordering the Straits of Malacca or the South China Sea (Figure 1). The farms represented a range of aquaculture systems that differed in culture practices, environmental conditions, and farm management. The range of aquaculture systems reflects variations in local geographical settings and available resources. To systematically analyse the impact of these farming practices on biological outcomes, the operations were classified into two open and semi-closed infrastructure systems. Additionally, three distinct farming methods

were identified: cage culture, earthen ponds, and fibreglass tanks. The rearing environment was characterised based on water source as either river, sea, or inland.

The open system refers to full exchange of water in the natural environment where the fish are contained. On the other hand, the semi-closed system refers to culture settings in a confined area, where exchange of water is dependent on the weather and man-power availability. For open systems operation, fish are **r**eared in floating net cages positioned within coastal seas or large river estuaries characterised by continuous, natural water exchange. The surveyed open-system sites include Tanjung Dawai, Bagan Lalang, and Sungai Marang. As for the semi-closed systems operation, fish are reared within confined inland infrastructures, such as earthen ponds or fibreglass tanks, where water exchange is intermittent and dependent on localised weather conditions and manual labour. The surveyed semi-closed sites include Kelanang, Gelang Patah, and Rompin.

Ambient salinity profiles during the field visits remained unaugmented and unmitigated across all sites, ranging natively between 21 and 27 ppt. For predictive modelling purposes, these sites were further partitioned into two explicit environmental categories based on baseline salinity thresholds of the low salinity group (≤ 25 ppt, comprising Kelanang, Bagan Lalang, and Gelang Patah) and high salinity group (> 25 ppt, comprising Tanjung Dawai, Sungai Marang, and Rompin).

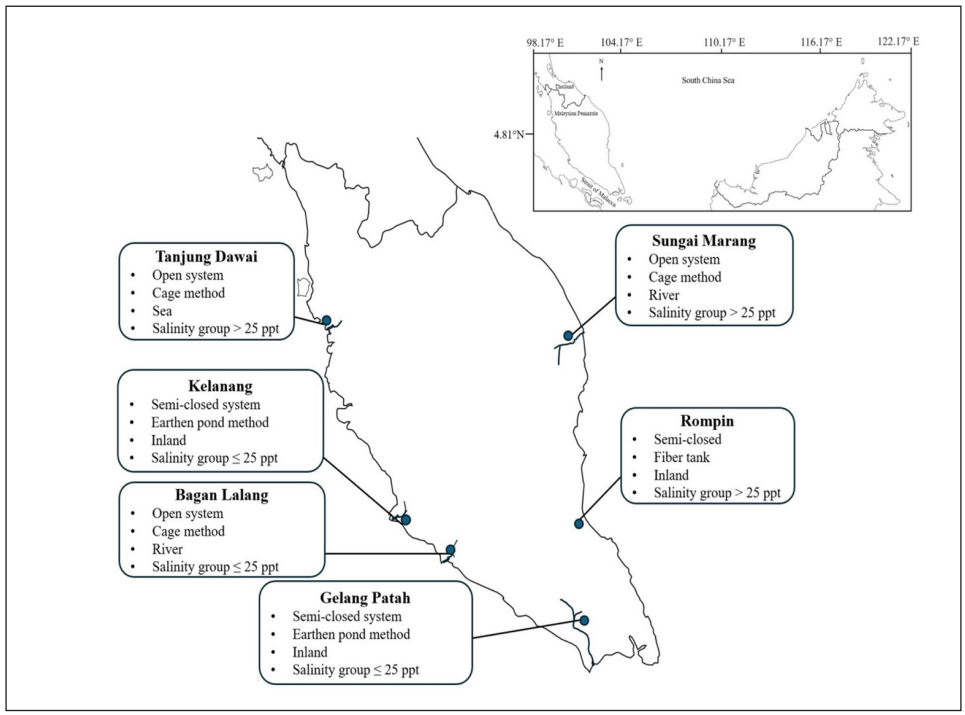


Figure 1. Sampling farms with their culture conditions

Fish Sampling and Biometric Growth Performance

Asian seabass was sampled (n = 112) from the six farms to obtain integrated biometric and histological profiling. At stocking, fingerlings had been introduced into their respective rearing systems at a standardised total length of approximately 5 inches. Because smallholding farmers did not maintain formal written historical registries, fish age (ranging from 5 to 42 months) was determined by calculating the duration between the farmer's recalled stocking date and the active sampling event. The fish were variably fed commercial pellet diets or available trash fish depending on age and individual farm resource constraints. Despite these differences, all farms followed a similar feeding schedule, with fish fed twice daily and feed rations adjusted according to fish body weight and culture duration. Variations in feeding practices among farms were considered an inherent limitation of this field-based study.

Individual fish ranged from 33 to 79 cm in total length (TL) and from 0.4 to 7.0 kg in body weight. We measured individual total lengths to the nearest 0.1 cm and recorded body weights to the nearest gram. From these metrics (Equations 1 to 4), we evaluated growth performance by calculating the length-weight relationship (LWR), relative condition factor (K), and specific growth rate (SGR).

- Length-weight relationship (LWR)

$$W = aL^b \quad [1]$$

- Logarithmic transformation to linear equation:

$$\log(W) = \log(a) + b \log(L) \quad [2]$$

where a denotes the intercept, and b denotes the allometric coefficient.

- Relative condition factor (K)

$$W = W_o/Wc \quad [3]$$

where W_o denotes observed body weight, and Wc denotes predicted body weight from the corresponding length-weight relationship.

- Specific growth rate (SGR, % day^{-1})

$$SGR = [(\ln W_2 - \ln W_1) / (T_2 - T_1)] \times 100 \quad [4]$$

where W_1 and W_2 are the initial and final body weights, respectively, and $(T_2 - T_1)$ is the culture duration in days. As stocking records were unavailable for all farms, an initial body weight of 100 g was assumed for all fish. Culture duration was estimated by converting the reported culture age from months to days using 30 days per month.

Microscopic Examination of Gonads

Following collection, fish were temporarily maintained in aerated 1,000-L holding tanks supplied with flow-through seawater until processing. Water temperature was maintained at 28-30 °C, salinity at 30-35 ppt, pH at 7.5-8.5, and dissolved oxygen above 6 mg/L. All procedures involving fish were conducted in accordance with the animal ethics guidelines approved by the Universiti Putra Malaysia Institutional Animal Care and Use Committee (UPM/IACUC/AUP-R010/2020).

Fish were euthanised by immersion in 200 mg/L clove oil solution (Sigma-Aldrich, CAS 8000-34-8). We confirmed death by verifying the absolute absence of reflex responses and opercular movement. Following that, we made a mid-ventral incision to expose the abdominal cavity, carefully harvesting the gonads with sterile instruments. To remove residual blood, we rinsed the excised tissues in 0.9% saline before fixing them in 10% neutral buffered formalin for 48 hours. The samples were then transferred to 70% ethanol for storage.

For histological processing, we cut the fixed gonads into roughly 2-cm sections and ran them through an automatic tissue processor on a standard 24-hour cycle. This sequence included dehydration via graded alcohol, xylene clearing, and final paraffin embedding. Finally, we sectioned the tissue blocks at 5-7 µm using a rotary microtome. Sectioned tissues were stained with hematoxylin and eosin (H&E) for microscopic evaluation.

Gonadal development was classified into four developmental stages following the modified criteria of Guiguen et al. (1994) and Terence et al. (2021). Stage 1 (Undifferentiated) comprised immature gonads showing no visible cellular differentiation. Stage 2 (Male) was characterised by an abundance of spermatogenic cells such as spermatocytes, spermatids, or mature spermatozoa in the absolute absence of advanced oocytes. Stage 3 (Intersex/ Transitional) displayed a mixture of both spermatogenic cells and developing primary or secondary oocytes. Lastly, Stage 4 (Female) was defined by the presence of secondary, vitellogenic, or hydrated oocytes with no remaining spermatogenic tissue.

Data Analysis

To check for data normality, we evaluated the skewness, kurtosis, and distribution of total length and body weight using Shapiro-Wilk tests alongside normal Q-Q plots. We also checked for homogeneity of variance across the farming sites using Levene's test before running any analysis of variance (ANOVA).

We modelled variations in the length-weight relationships among the farms using a General Linear Model (GLM). To test for divergence in regression slopes, we evaluated the interaction term between farm site and log-transformed total length via ANOVA, an approach functionally equivalent to an analysis of covariance (ANCOVA). For each farm, the estimated allometric coefficient (b) was further compared with the theoretical value for isometric growth ($b = 3$) using a two-tailed one-sample t-test. Failure to reject the null hypothesis (H_0: $b = 3$) indicates isometric growth, whereas significant deviations indicate either positive or negative allometric growth.

Variations in somatic parameters (total length and body weight) across the four ordinal stages of gonadal development were assessed via one-way ANOVA. Post-hoc comparisons were executed using Tukey's Honest Significant Difference (HSD) test, with the *a priori* significance level at $\alpha = 0.05$.

Relative condition factor, K and specific growth rate (SGR) were then evaluated for their differences across farms. Data normality was first assessed using the Shapiro-Wilk test. Both K and SGR data violated normality assumptions ($p < 0.001$), and transformations (log, square root, Box-Cox) failed to achieve normality (all $p < 0.05$). Therefore, non-parametric tests were employed. Differences in K and SGR between farming systems, i.e. open (Bagan Lalang, Marang and Tanjung Dawai) vs. semi-closed (Gelang Patah, Kelanang and Rompin) were compared using the Mann-Whitney U test. Comparisons among farming methods (cage, earthen pond, fibre tank; $n = 3$ groups) and environments (river, sea, inland; $n = 3$ groups) were not conducted due to insufficient sample sizes within several groups (e.g., only one farm representing cage-river system with n = 10, and one farm representing cage-sea system with n = 10), which would compromise statistical power and increase the risk of Type II errors. Additionally, the unequal distribution of samples across method and environment categories violated the assumptions of multi-group non-parametric tests. Therefore, analyses were restricted to comparisons between the two farming systems (open vs. semi-closed; n = 30 and n = 27, respectively), which provided adequate and balanced sample sizes for robust statistical inference. For comparisons across multiple farms, the Kruskal-Wallis test was used, followed by Dunn's post-hoc test with Bonferroni correction for pairwise comparisons. Effect sizes were calculated as eta-squared (η^2).

To determine which farm characteristics best explained variation in gonadal development, we executed model selection via an information-theoretic approach. This framework utilised Akaike's Information Criterion corrected for small sample sizes (AICc). Six candidate ordinal logistic regression models were developed using different combinations of three categorical predictors: culture method (cage, earthen pond, or fibre tank), rearing environment (river, sea, or inland), and farming system (open or semi-closed). Gonadal development stage was used as the ordinal response variable (undifferentiated = 1, male = 2, intersex = 3, and female = 4). Candidate models were compared using AICc, the

difference in AICc from the best-supported model (ΔAICc), and Akaike weights (AICcWt), which indicate the relative support for each model. Cumulative Akaike weights were also calculated to assess the overall importance of the candidate models. Log-likelihood values from each fitted model were used to calculate AICc.

Ordinal logistic regression (OLR) was then used to examine the relationship between fish size and gonadal development. Total length (TL) was included as the explanatory variable, while gonadal development stage was treated as the ordinal response. Body weight was excluded because it was highly correlated with total length (r = 0.94). This was confirmed by variance inflation factor (VIF) analysis, and excluding body weight reduced multicollinearity in the model.

A second OLR model was fitted using only grow-out fish (≤ 9 months old) to estimate the probability of precocious male differentiation under different rearing environments. To maintain statistical clarity, we used p exclusively for hypothesis testing significance levels and reserved $\hat{p}$ to signify the predicted probabilities output by the ordinal logistic regression models. All statistical analyses and graphical presentations were performed in RStudio (RStudio Team, 2020).

RESULTS

Fish Growth Performance

The length-weight relationship revealed variation in growth patterns among farms, with b values ranging from 2.12 to 3.59. Except for Bagan Lalang, all of the surveyed populations showed no significant deviation from the theoretical isometric value ($b = 3$), maintaining a proportional weight increase relative to length across the different farming systems. The only anomaly that occurred at Bagan Lalang showed that the fish exhibited distinct negative allometric growth ($b < 3$).

Growth patterns varied among the farming sites, with calculated b values spanning from 2.12 to 3.59 within the length-weight relationships. Most populations showed no meaningful deviation from the theoretical isometric value ($b = 3$), tracking a proportional increase in body weight alongside length across the different systems. The sole exception was Bagan Lalang, where the fish displayed distinct negative allometric growth ($b < 3$)

The general linear model accounted for 98.5% of the variation in log-transformed body weight ($R^2 = 0.9853$). Model residuals showed no significant deviation from normality (Shapiro-Wilk test: $W = 0.9835$, $p = 0.626$) and no evidence of heteroscedasticity based on the Breusch-Pagan test ($\chi^2 = 17.17$, $p = 0.103$). The ANCOVA revealed a significant log Total Length × farm interaction ($F_{5, 110} = 3.82$, $p = 0.006$), indicating that the growth exponent b in the power equation $W = aL^b$ varies among farms (Table 1). Post-hoc comparisons further revealed that only the Bagan Lalang-Marang slope contrast was significant ($p = 0.009$), with all other pairwise comparisons non-significant ($p > 0.05$).

The Mann-Whitney U tests revealed statistically significant differences in both relative condition factor (*K*) and specific growth rate (SGR) between open and semi-closed farming systems. For *K*, the median was 1.18 (interquartile range, IQR = 1.07-1.25) in open systems and 1.30 (IQR = 1.09-1.48) in semi-closed systems, respectively (W = 172, $p < 0.001$, r = 0.49). For SGR, the median was 0.99 (IQR = 0.49-1.14) in open systems and 1.01 (IQR = 0.93-1.16) in semi-closed systems, respectively (W = 279.5, $p = 0.046$, r = 0.27). Semi-closed systems exhibited higher central values and reduced variability for both parameters, as indicated by narrower IQR and smaller standard deviations. The effect size for *K* ($r = 0.49$) was nearly double that of SGR ($r = 0.27$), indicating a more pronounced system-related difference in condition factor than in growth rate.

Gonad Development by Means of Histological Examination

Microscopic examination showed clear differences in gonadal structure among the developmental stages. Histological examination of the male gonads revealed a clear dominance of spermatogenic cells arranged within distinct tubular structures, staining a vivid blue purple (Figure 2a). Staining intensity shifted dynamically with spermatogenic stages. Under 10× magnification, spermatocytes stood out as loosely organised circular profiles with clearly visible nuclei. In contrast, mature spermatozoa clustered tightly within the seminiferous tubules, and we noted a complete absence of oogonia in these male-stage tissues.

The intersex gonads presented a complex cellular mosaic, where male and female germ lines, including oogonia, previtellogenic oocytes, and vitellogenic oocytes, were observed at varying developmental depths, with the presence of atretic oocytes and residual spermatogenic cells (Figure 2b). We observed wide structural variation across this transitional cohort, ranging from gonads heavily anchored by spermatogenic tissue with sparse oocytes to specimens overflowing with oocytes alongside dying spermatogenic clusters.

Developing oocytes at secondary or advanced vitellogenic stages indicated fully developed female gonads. The ovaries displayed asynchronous development, showcasing multiple developmental phases simultaneously to give the tissue a signature pink-to-red appearance (Figure 2c). Follicular thickness and yolk accumulation increase as vitellogenic oocytes expand in size. Where atretic oocytes occurred, we identified them by their irregular boundaries, disrupted cellular layout, and depleted yolk volume.

All four stages of Asian seabass gonadal development were present within our sampled cohorts upon histological evaluation. Most individuals were classified as undifferentiated (66.1%), while the remaining cohort consisted of either males (17.9%), females (8.9%), or intersex individuals (7.1%). Fish collected from the Kelanang, Sungai Marang, and

Table 1

Descriptive statistics and growth performance metrics of Asian seabass sampled across six Malaysian aquaculture farms, where ± for b indicates mean and standard error, while for K and SGR indicate median and interquartile range (IQR)

Farm	n	Weight, g	Total length, cm	Age (Months)	b	a	Relative Condition Factor, K	SGR, % day^{-1}
Bagan Lalang	18	922 ± 290 [a]	45.0 ± 12.6[a]	7	2.12 ± 0.24[a]	0.33	1.22 ± 0.24	0.97 ± 0.26
Gelang Patah	20	3,940 ± 1,429 [b]	65.5 ± 7.9[b]	30 - 42	2.89 ± 0.23[ab]	0.02	1.29 ± 0.11	0.34 ± 0.04
Kelanang	14	601 ± 77 [a]	35.9 ± 1.2[a]	5	3.28 ± 0.91 [ab]	0.00	1.21± 0.08	1.15 ± 0.09
Sungai Marang	20	1,460 ± 503 [a]	48.6 ± 4.4[a]	7	3.59 ± 0.43 [b]	0.00	1.22 ± 0.15	1.25 ± 0.16
Rompin	20	1,750 ± 458 [a]	48.0 ± 4.6[a]	9	2.82 ± 0.19 [ab]	0.03	1.55 ± 0.09	1.05 ± 0.10
Tanjung Dawai	20	565 ± 235 [a]	36.7 ± 4.5[a]	6	2.85 ± 0.21 [ab]	0.02	1.10 ± 0.08	0.93 ± 0.18

Note. Values expressed with ± indicate the mean ± standard deviation. Coefficients a = intercept and b = slope of the log-transformed length-weight equation, K = relative condition factor, and SGR = specific growth rate. Different superscript letters for Weight, Total length and b indicate statistically significant differences between farms (one-way ANOVA $p < 0.05$) based on the Tukey-HSD post-hoc test

Tanjung Dawai farms were exclusively at the undifferentiated stage, whereas the other farms contained individuals representing multiple stages of gonadal development (Figure 3). The occurrence of different developmental stages within the same farm indicates variation in the timing of sexual differentiation among cultured fish.

Across the sampled population, our histological screening captured all four stages of gonadal development in the Asian seabass. Most individuals were undifferentiated (66.1%), with the remaining cohort consisting of males (17.9%), females (8.9%), and intersex variants (7.1%). Interestingly, sampling sites varied in maturity; fish from Kelanang, Sungai Marang, and Tanjung Dawai were entirely undifferentiated. By contrast, the remaining farms yielded mixed populations spanning multiple reproductive stages (Figure 3).

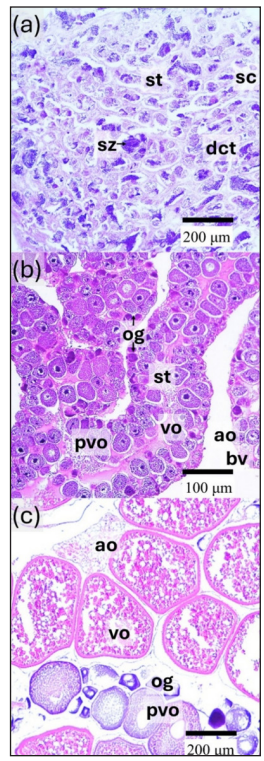


Figure 2. Histological sections of Asian seabass gonads stained with hematoxylin and eosin (H&E): (a) male (67 cm TL, 3.9 kg body weight), (b) intersex (56 cm TL, 2.4 kg body weight), and (c) female (72 cm TL, 4.6 kg body weight). All images were taken at 10× magnification. st = spermatids, sc = spermatocytes, sz = spermatozoa, pvo = previtellogenic oocytes, vo = vitellogenic oocytes, ao = atretic oocytes, og = oogonia, dct = dense connective tissue, and evo = early-stage vitellogenic oocyte

The relationship between somatic growth and gonadal development showed considerable variation among individuals. Across the sampled size range (56-79 cm TL and 2.2-7.0 kg body weight), fish of similar size were observed at different stages of gonadal development (Figure 4). Gonadal stages did not follow a consistent progression according to body size, as undifferentiated, male, intersex, and female individuals occurred across overlapping size ranges. For example, undifferentiated and intersex fish were both observed at 56 cm TL, while males occurred across a wide body weight range (2.4-7.0 kg). Similarly, fish with the same total length (e.g., 72 cm) were classified as either intersex or female based on histological examination.

Size Overlap and Correlation Analysis

Body size varied considerably among the different gonadal developmental stages. Male individuals ($n = 20$) ranged from 41.5 to 72 cm TL and 0.95 to 4.6 kg body weight. Intersex fish ($n = 8$) ranged from 56 to 73 cm TL and 2.2 to 4.8 kg, whereas females ($n = 10$) ranged from 62 to 79 cm TL and 2.8 to 7.0 kg.

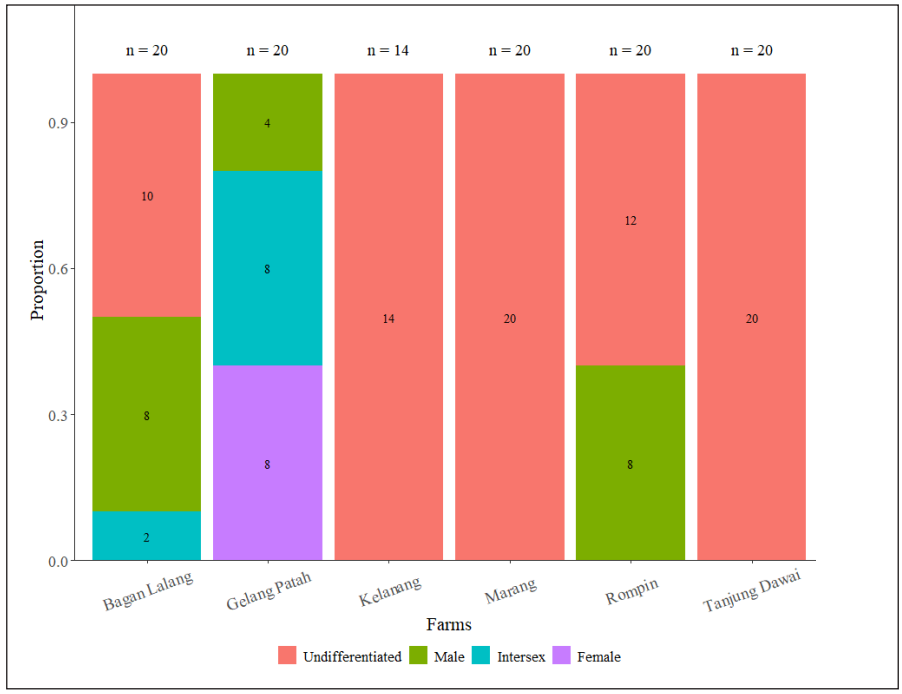


Figure 3. Proportion of gonadal developmental stages among Asian seabass sampled in this study. Numbers within each section of the stacked bars represent the number of individuals

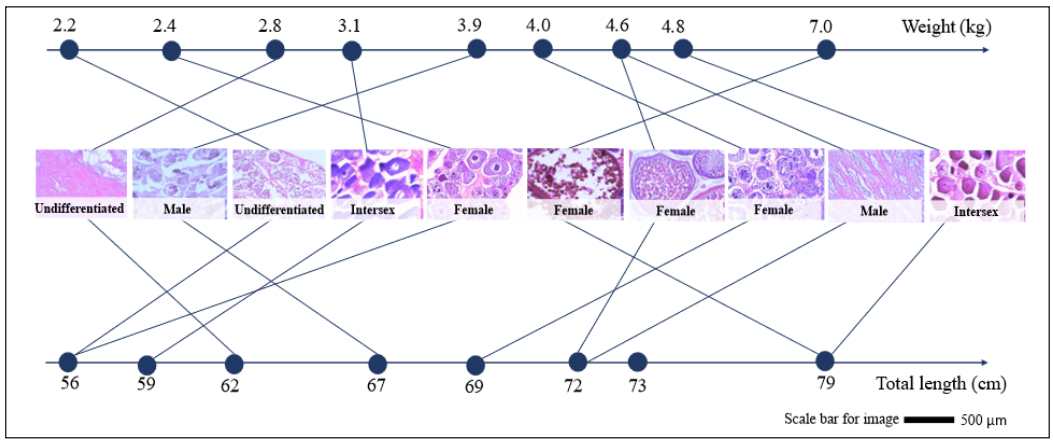


Figure 4. Relationship between gonadal developmental stages and body size (total length and body weight) of Asian seabass. Representative histological images at 20× magnification show variation in gonadal development among individuals of different size classes

A strong positive relationship between total length and body weight was observed within each farm, with Pearson's correlation coefficients exceeding 0.86 (Figure 5). Similar length-weight patterns were observed among farms, suggesting comparable somatic growth trends across the surveyed operations. When all samples were pooled, total length and body weight showed a strong correlation ($r = 0.94$), indicating that both measurements provided similar information regarding fish size.

One-way ANOVA showed significant differences in biometric characteristics among gonadal developmental stages. Intersex and female individuals were significantly larger than male and undifferentiated fish ($p < 0.01$; TL: $F_{3,33} = 13.75$; weight: $F_{3,33} = 10.42$) (Figure 6). A variance inflation factor (VIF) diagnostic confirmed strong collinearity between total length and body weight (VIF = 8.59), prompting us to retain only the former for subsequent regression modelling.

Ordinal Logistic Regression Models

The AICc-based model selection indicated that the combination of culture method and rearing environment provided the strongest support for explaining variation in gonadal development (ΔAICc = 0; Akaike weight = 0.26) (Table 2). Among the candidate models, those including rearing environment accounted for most of the model support, with a cumulative Akaike weight of 0.76. In contrast, models without rearing environment received substantially lower support (ΔAICc > 2). *Method* + *Environment* ranks as the top model (AICc = 108.78, ΔAICc = 0, weight = 0.26), followed by *Environment* alone (AICc = 108.88, ΔAICc = 0.1) and *System* + *Environment* (AICc = 108.88, ΔAICc = 0.1) rank joint second. All models have *K* values between 4 and 6 parameters, with similar log-likelihood scores ranging from -48.2 to -51.52. The ΔAICc differences between the top three models are less than 2, indicating they have substantial empirical support. Models containing *Environmen*t that records farming practices at sea, in the river and inland, consistently appear in the top three positions. Models without *Environment* show ΔAICc values exceeding 2, indicating considerably less support.

Exponentiating the logit coefficient values provided odds ratios (OR) that explain how the predictors vary with the response. Holding environmental salinity constant, fish exhibited a 1.2-times increase in the odds of shifting into advanced stages of gonadal development for every 1 cm increase in total length (OR = 1.2 ± 0.6 SE; Figure 7a). Conversely, when keeping total length constant, the gonads demonstrated 9.1 times higher odds of being in advanced developmental stages if the fish were cultured in a low-salinity environment (OR = 9.1 ± 1.0 SE).

The predicted probability ($\hat{p}$) curves indicated that while the likelihood of transitioning into a female increases with total length, the speed of this transition depends heavily on salinity (Figure 7b). Given the same TL, fish cultured in low salinity had a higher

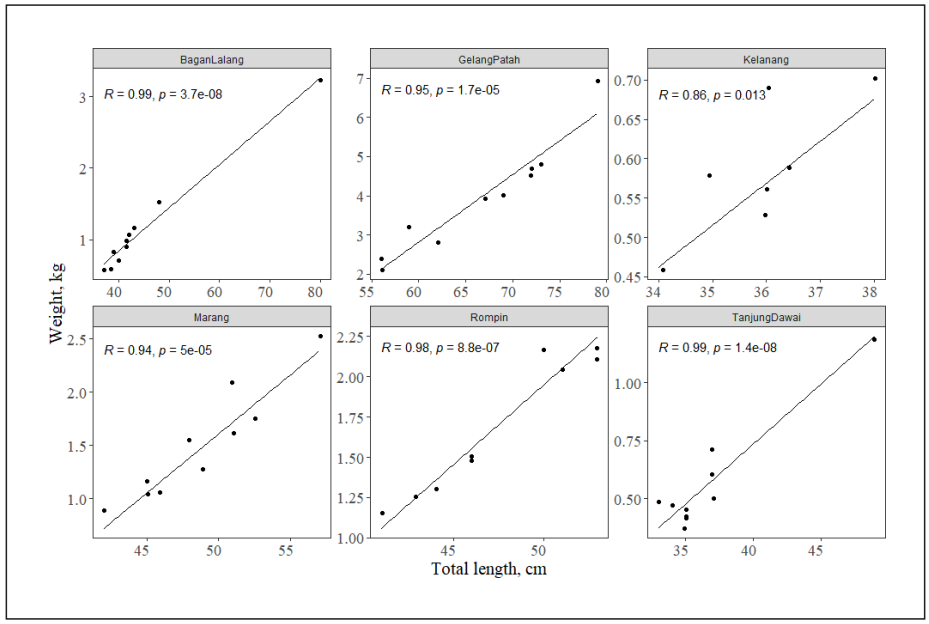


Figure 5. Pearson correlation between total length and body weight of Asian seabass sampled from each farm

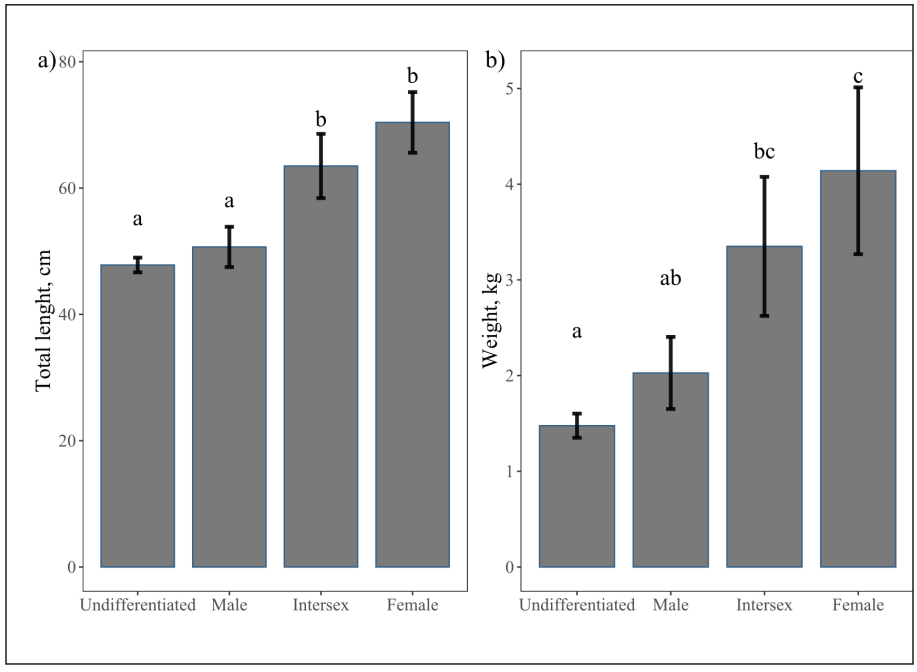


Figure 6. Comparison of Asian seabass size according to gonadal developmental stage: (a) total length and (b) body weight

Table 2
Corrected Akaike Information Criterion (AIC_c) model ranking of farm infrastructure effects on Asian seabass sex reversal

Model	K	AIC_c	ΔAIC_c	AIC_cW_t	Cumulative W_t	Log-likelihood
Method + Environment	6	108.78	0	0.26	0.26	-48.2
Environnment	5	108.88	0.1	0.25	0.51	-49.3
System + Environment	5	108.88	0.1	0.25	0.76	-49.3
Method	5	111.12	2.34	0.08	0.84	-50.43
System + Method	5	111.12	2.34	0.08	0.92	-50.43
System	4	111.22	2.44	0.08	1	-51.52

Note. K = number of estimated model parameters (including intercepts); AIC_c = Akaike Information Criterion corrected for small sample sizes; ΔAIC_c = difference in ΔAIC_c value relative to the top-ranked model; AIC_cW_t = Akaike model weight indicating relative predictive probability; Cumulative W_t = running sum of model weights within the candidate set; Log-likelihood = maximised log-likelihood value of the fitted regression function. System denotes structural infrastructure class (open vs. semi-closed), Method denotes rearing technique (cage, pond, or tank), and Environment denotes the baseline salinity classification (≤ 25ppt vs. > 25 ppt)

predicted probability ($\hat{p}$) of transitioning to an advanced gonadal stage than fish cultured in high salinity. For example, the gonads began to differentiate into functional males earlier when cultured in low salinity than high salinity, achieving a peak predicted probability of $\hat{p}$ = 0.60 at 52 cm TL compared to 61 cm TL, respectively. Similarly, the total length at which a fish crossed a $\hat{p}$ > 0.50 probability of becoming female was reached at approximately 73 cm TL under low salinity but was delayed until 82 cm TL under high salinity regimes.

Age-based regression models confirmed that fish also exhibited 1.2 times increase in the odds of occupying advanced stages of gonadal development for each additional month of age (OR = 1.2 ± 0.6 SE; Figure 8a). The probability curves showed that the likelihood of transitioning into a female increases steadily with age (Figure 8b). At 40 months of age, the probability of an individual developing as

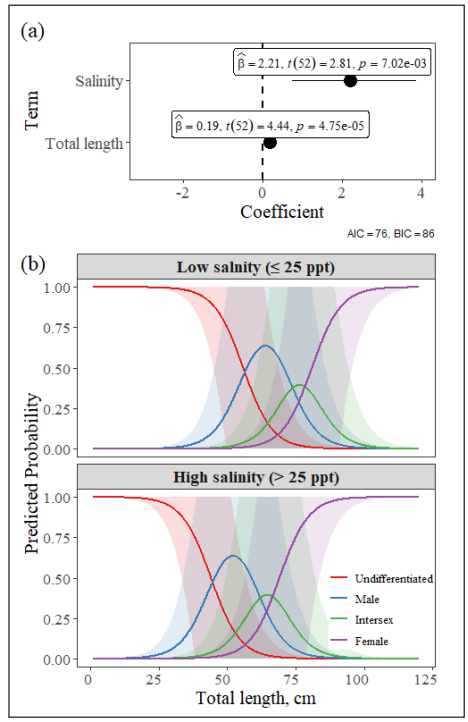


Figure 7. Predicted proportions and confidence intervals of (a) Parameter estimates and (b) respective predicted probability of Asian seabass sexually differentiated at varying gonad stages in low (≤ 25 ppt) and high (> 25 ppt) high range of salinity rearing environments

a female within the Gelang Patah cohort reached $\hat{p}$ = 0.75. By this 40-month threshold, the total probability of a fish possessing a differentiated gonad (male, intersex, or female) reached 100%. The active intersex transition window peaked at 34 months of age with a maximum predicted probability of $\hat{p}$ = 0.45 (Figure 8b). Prior to this transition, younger fish reached a $\hat{p}$ = 0.70 probability of functioning as males at approximately 20 months of age. As age progressed, the probability of being male at 22 months of age was maintained at $\hat{p}$ = 0.70. Following the 22-month milestone, the fish had a higher probability of entering the transition phase, which peaked at $\hat{p}$ = 0.45 at 36 months of age. The narrow age band and low overall probability curve for the intersex stage across the models confirm that this transitional stage is short-lived.

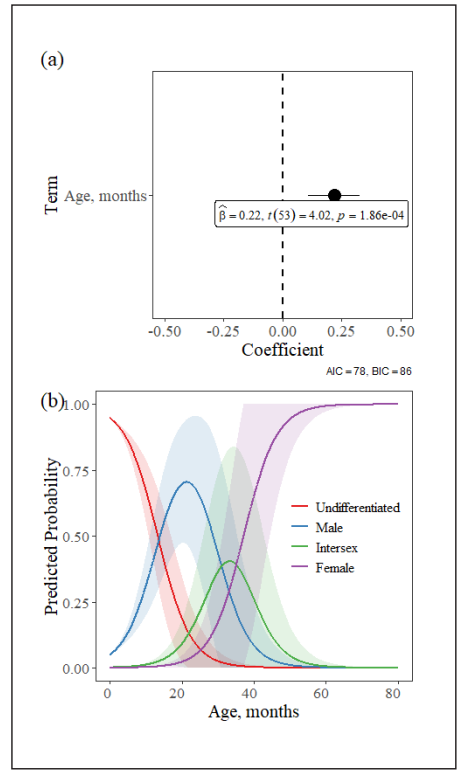


Figure 8. Predicted proportions and confidence intervals of (a) Parameter estimates and (b) respective predicted probability of Asian seabass sexually differentiated with age

For the condition factor model, the logit coefficient was not significant (p = 0.13), suggesting that *K* alone was not a strong predictor of gonadal development (Figure 9). Across the observed range of *K* values (0.6-1.7), the predicted proportion of undifferentiated fish gradually decreased, whereas the probabilities of male, intersex, and female stages showed only small increases. In contrast, the SGR model showed a significant relationship with gonadal development (p = 0.02). As SGR increased, the probability of undifferentiated fish decreased, accompanied by an increase in the probability of male development followed by higher probabilities of intersex and female stages at greater growth rates.

Model comparisons confirmed that both the condition factor (AIC = 119) and SGR (AIC = 115) frameworks offered substantially weaker predictive power. Their elevated AIC scores fell short of the superior total length (AIC = 76) and chronological age (AIC = 84) models. Our findings show that total length (as a measure of structural size) and chronological age are far more reliable predictors of gonadal development than condition factor or SGR.

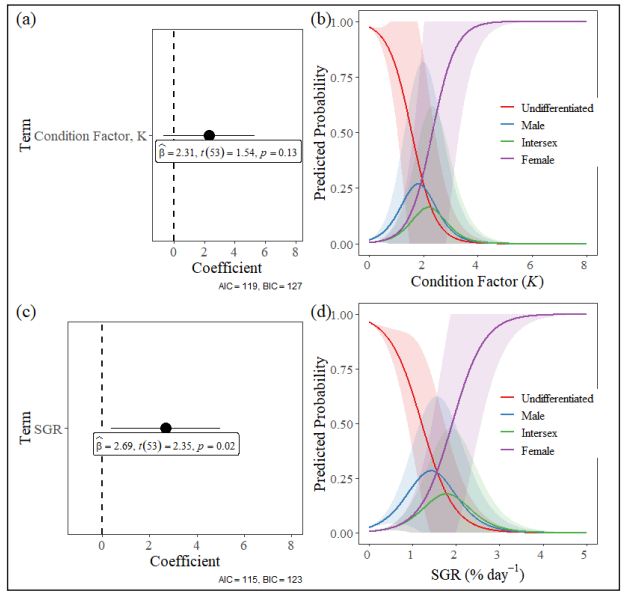


Figure 9. Predicted proportions and confidence intervals for (a) condition factor (K) parameter estimates, (b) predicted proportions based on K, (c) specific growth rate (SGR) parameter estimates, and (d) predicted proportions based on SGR

DISCUSSION

The length-weight relationship indicated predominantly isometric growth across the evaluated Asian seabass farming systems, except for Bagan Lalang, as fish from the farm showed negative allometric growth. The findings contrasted previous reports of positive allometric growth in Asian seabass under favourable culture conditions (Asres et al., 2023). Isometric growth reflects proportional increases in length and weight, which does not necessarily indicate poor growth performance (Che Abdullah & Md Zain, 2019). In terms of performance, all farms maintained favourable condition factors (K = 1.10-1.55) and SGR values (0.34-1.25% day^{-1}) that reflect good overall growth status.

The low b value at Bagan Lalang may be related to its younger age structure, where energy allocation may favour length increase before greater body mass accumulation (Froese, 2006). Given the protandrous hermaphroditic nature of Asian seabass, these variations in b exponents among the sites reflect localised differences in culture conditions alongside shifting ontogenetic and maturation stages (Sari et al, 2020; Kumar et al., 2016).

Cage culture is the dominant production system in Malaysia, followed by pond culture; each system presents different advantages and constraints. For example, a closed aquaculture system with salinity maintained between 20 and 36 ppt has been reported to achieve an optimal SGR of 8.74 ± 0.03 % day^{-1} (Hassan et al., 2024). However, previous studies have also shown that different culture systems produce comparable performance among fry, fingerlings (Sorphea et al., 2019), and grow-out fish (Szentes et al., 2012).

The present study also found that infrastructure type alone did not result in differences in growth patterns, as fish from all surveyed farms exhibited isometric growth. This suggests that cultured Asian seabass were able to maintain proportional increases in length and weight under different farming conditions. Similar growth patterns may reflect effective energy allocation towards maintaining body condition and adaptation to local culture environments (Dikou, 2023; Mitra et al., 2023; Zhang et al., 2023). In contrast, allometric growth patterns have been reported in some marine cage-cultured populations (Che-Zulkifli et al., 2023), where growth allocation may vary according to environmental conditions, age, and reproductive status (Athauda & Anderson, 2013). Juvenile fish frequently skew toward positive allometry during rapid physical expansion. Conversely, as fish mature, shifting metabolic priorities often redirect energy away from immediate mass gain, driving older individuals toward isometric or negative allometric growth patterns (Froese, 2006; Lupatsch et al., 2003).

Operational profitability in aquaculture relies heavily on optimising growth performance. In semi-closed earthen pond systems, growth rates often fluctuate due to shifting environmental variables, organic loading, algal blooms, and erratic oxygen levels. Managing these volatile dynamics requires daily control over pH, dissolved oxygen, and ambient ammonia (Abd El-Hack et al., 2022; Aljehani et al., 2023). Open cage systems benefit from continuous water exchange and natural oxygenation, which can support higher stocking densities, although these systems remain vulnerable to external environmental changes such as pollution and harmful algal blooms (Price et al., 2014; Soto et al., 2008).

Although feeding practices differed among farms, including the use of commercial pellets or trash fish depending on fish size and farm resources, all farms followed a similar feeding frequency of twice daily. Feed quantities were adjusted according to estimated fish biomass and culture duration. Differences in feed type among farms represent a limitation of this field survey; however, the consistency in feeding frequency may have reduced variation in feeding-related management practices among farms.

CONCLUSION

This study indicates that small-scale aquaculture systems in Malaysia can support stable growth patterns in Asian seabass (*Lates calcarifer*) but may also be associated with early onset of male differentiation during the grow-out phase. Condition factor (K) was comparable among culture systems, whereas fish reared in semi-closed systems exhibited higher specific growth rates than those cultured in open floating cages. However, ordinal logistic regression and AIC_c-based model selection proved that environmental salinity remains the primary driver of reproductive transitions. Holding body length constant, fish in low-salinity environments (≤ 25 ppt) exhibit 9.1 times higher odds of entering advanced gonadal stages, compressing the 50% female transition threshold down to 73 cm from 82

cm TL under high-salinity regimes (> 25 ppt). Consequently, grow-out operations utilising semi-closed systems must prioritise strategic water-source sourcing or timed high-salinity water intake to prevent premature maturation from diverting metabolic energy away from muscle production. Conversely, broodstock managers can leverage low-salinity configurations to accelerate female sex-reversal timelines. These operational applications optimise commercial farming predictability. However, future longitudinal research under controlled laboratory conditions is required to overcome unavoidable field constraints. Such limitations include unmonitored daily water chemistry parameters and limited late-stage sample sizes.

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Influence of Culture Media on Growth and Conidial Quality of Two *Trichoderma* spp. Isolates with Preliminary Evaluation Against Downy Mildew in Maize

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ABSTRACT

Downy mildew caused by *Peronosclerospora* spp. is a major disease of maize (*Zea mays* L.), and *Trichoderma* spp. are widely used as biological control agents against this pathogen. This study aimed to determine the effect of two *Trichoderma* spp. isolates propagated on different media on the incidence of downy mildew in maize. The study was conducted at the Biological Control Agent Laboratory of the West Java Plantation Protection Agency and in a field in Pasirbiru Village, Rancakalong District, Sumedang Regency, from April to May 2025. A Completely Randomised Factorial Design with two factors, isolate type (*Trichoderma* spp. from BBPOPT and BBPPTP) and propagation medium (cracked corn and rice bran), replicated five times, was used in the laboratory. At the same time, a Randomised Block Design was applied in the field to evaluate effectiveness against downy mildew. The propagation medium significantly affected the reduction in medium weight before and after inoculation, with rice bran reducing medium weight by up to 1.20 g. The BBPOPT and BBPPTP isolates grown on maize medium produced the lowest numerical AUDPC values (128.33 and 122.50, respectively) and the highest numerical disease suppression (42.8% and 45.40%, respectively), although differences among treatments were not statistically significant. These results indicate that *Trichoderma* spp. treatments delayed early-stage disease development but did not significantly suppress overall downy mildew progression. As field evaluation was conducted at only one location and season, further multi-location, multi-season studies are needed to confirm the consistency of these effects

Keywords: Bran, conidial viability, conidial density, corn, *Peronosclerospora* spp., *Trichoderma* spp. isolates

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INTRODUCTION

Downy mildew is one of the most important diseases affecting maize. This disease is caused by several species belonging to the genera *Peronosclerospora* and *Sclerospora* (Muis et al., 2016; Rashid et al., 2013). Yield losses caused by downy mildew in maize, particularly in susceptible varieties, may reach 90-100% (Nirwanto & Sutikno, 2024). Such high yield losses can result in significant economic damage if the disease is not properly managed.

The most common method for controlling downy mildew is the application of synthetic chemical fungicides. Fungicides containing metalaxyl as the active ingredient are widely used for controlling downy mildew (Prasetyo et al., 2021). However, continuous application without proper dosage consideration may lead to several negative impacts. Pesticides can contaminate the environment and non-target organisms, including beneficial soil microorganisms, and their residues may pollute soil and water resources (Aktar et al., 2009). Excessive pesticide application may also cause residue accumulation in agricultural products, posing risks to human health (Kurniasih et al., 2013). Furthermore, studies by Widiantini et al. (2017) indicated the emergence of resistance to metalaxyl fungicides in *Peronosclerospora* spp. Therefore, environmentally friendly alternative control strategies are required to reduce the negative impacts of synthetic pesticides.

One alternative control strategy is the use of biological control agents (BCAs). BCAs are living organisms that can suppress plant pests and diseases by predation or parasitism (Muley & Chavan, 2023). One of the potential BCA is *Trichoderma* spp., a saprophytic fungus commonly found in soil, air, and plant surfaces (Gusnawaty et al., 2017; Wang et al., 2022; Yao et al., 2023). *Trichoderma* spp. suppress plant diseases through several mechanisms, including antibiosis, competition for space and nutrients, and parasitism (mycoparasitism or hyperparasitism) (Gusnawaty et al., 2014; Prasad et al., 2023). In addition, *Trichoderma* spp. can indirectly induce plant resistance and promote plant growth (Nakkeeran et al., 2018).

Previous studies reported that *Trichoderma* spp. effectively controlled several plant pathogens, such as clubroot in pak choi, white root rot in rubber seedlings, and vascular streak dieback (VSD) in cocoa seedlings (Amaria & Wardiana, 2014; Harni et al., 2017; Yudha et al., 2016*). Trichoderma* spp. have also been reported as biological control agents against insect pests (Poveda, 2021). Isolate *T. harzianum* T019 increased ergosterol production, which induced defence-related genes in common bean against *Rhizoctonia solani* infection (Mayo et al., 2015). The combination of *T. asperellum* and betel leaf extract was reported to suppress downy mildew incidence in maize (Prasetyo et al., 2021).

The physiological quality of *Trichoderma* spp. inoculum, such as conidial density and viability, which can influence characteristics and the propagation media, is considered an important factor influencing its potential performance as a biological control agent. *Trichoderma* spp. require carbohydrates, proteins, and other nutrients to support their

growth (Jaya et al., 2023; Novianti, 2018). To date, maize and rice are commonly used as substrates for mass propagation of *Trichoderma* spp. However, these substrates are relatively expensive, necessitating the use of alternative, more affordable, and readily available propagation media. Several studies reported that rice, maize, rice bran, rice husk, sawdust, sweet potato, cassava, bran, potato, tofu waste, and millet can be used as substrates for mass propagation of *Trichoderma* spp. (Gusnawaty et al., 2017; Kansrini, 2015; Novianti, 2018; Safitri et al., 2023; Suharni et al., 2023; Utami et al., 2023). Therefore, this study evaluated two *Trichoderma* spp. isolates propagated on different media for their effectiveness in reducing downy mildew (*Peronosclerospora* spp.) incidence in maize.

MATERIALS AND METHODS

This study was conducted using an experimental method at two locations: the Biological Control Agent Laboratory of the West Java Plantation Protection Agency and a field site in Pasirbiru Village, Rancakalong District, Sumedang Regency, at an altitude of 850 metres above sea level. The experiment was conducted from April to May 2025.

Laboratory equipment included a digital balance, beakers, cooking pots, stirrers, stove, Petri dishes, inoculating needles, Bunsen burner, steamer, wooden spatula, trays, electric fan, glass jars, cork borer, test tubes, micropipettes, glass funnels, test tube racks, Laminar Air Flow cabinet (LAF), autoclave, Erlenmeyer flasks, vortex mixer, hemocytometer, hand counter, syringe, microscope slides, cover slips, scalpel, and microscope. Field equipment included 10 kg polybags, stakes, and hoes.

Materials used in the Laboratory included pure cultures of *Trichoderma* spp. obtained from the Indonesian Plant Protection Forecasting Center (BBPOPT) Karawang and the Center for Seed and Plantation Crop Protection (BBPPTP) Surabaya, sterile distilled water, Potato Dextrose Agar (PDA), 70% alcohol, spiritus, toothpicks, cotton, tissue paper, and propagation media consisting of cracked maize and rice bran. Field materials included maize seeds of the Bisma variety, planting media (rice husk, manure, and soil), and maize plants infected with downy mildew.

Experimental Design

Laboratory Experiment

The laboratory experiment employed a Completely Randomised Design Factorial pattern with two factors. The first factor was the *Trichoderma* spp. isolate, consisting of BBPOPT isolate (t_1) and BBPPTP isolate (t_2). The second factor was the propagation medium, consisting of cracked maize (m_1) and rice bran (m_2). Each treatment was replicated five times, with two Petri dishes per replication, resulting in 40 experimental units. Observations included fungal growth parameters (incubation period and medium weight loss before and after incubation) and conidial quality parameters (conidial density and viability).

Field Experiment

The field experiment was arranged in a Randomised Block Design (RBD). Treatments consisted of combinations of two *Trichoderma* spp. isolates propagated on different media that had previously been tested under laboratory conditions to evaluate their effectiveness against downy mildew incidence. Five treatments were applied: A = control, B = t_1m_1 (BBPOPT isolate on cracked maize), C = t_1m_2 (BBPOPT isolate on rice bran), D = t_2m_1 (BBPPTP isolate on cracked maize), and E = t_2m_2 (BBPPTP isolate on rice bran). Each treatment was replicated five times. Each replication consisted of 16 plants grown in two polybags, resulting in a total of 400 observed plants. Observations included downy mildew incidence and the Area Under the Disease Progress Curve (AUDPC).

Propagation of **Trichoderma** *spp. Isolates on PDA Medium*

The *Trichoderma* spp. isolates utilised in this experiment were first propagated on Potato Dextrose Agar (PDA) medium. PDA is a commonly used medium for culturing various fungi and supports mycelial growth effectively (Devi et al., 2018). The PDA medium used was instant PDA, prepared by dissolving 42 g of PDA powder in 1 L of distilled water in a beaker. The solution was heated using the double-boiling method until boiling. The boiled medium was subsequently transferred to an Erlenmeyer flask and sterilised in an autoclave at 121 °C for 45 minutes under 1 atm. Following sterilisation, approximately 10 mL of the medium was aseptically dispensed into Petri dishes.

After solidification, the PDA medium was inoculated with *Trichoderma* spp. within a Laminar Air Flow (LAF) cabinet that had been sterilised using 70% alcohol and ultraviolet light for 15 minutes. Inoculation was performed by transferring an agar plug containing mycelia and spores of *Trichoderma* spp. from an actively growing culture to a fresh PDA medium using an inoculating needle. The inoculated cultures were incubated for approximately 7-14 days, or until the fungal mycelium evenly covered the medium surface.

Propagation of Trichoderma spp. on Cracked Maize and Rice Bran Medium

The preparation of cracked maize medium for *Trichoderma* spp. propagation followed the method commonly used by BPP. The maize grains were washed, steamed until partially cooked, and then air-dried using a fan. Meanwhile, the rice bran medium was prepared according to the method described by Day et al. (2022), with modifications. Rice bran was mixed with water at a 1:2 ratio until the moisture content reached approximately 30%, as indicated by the absence of free water release and the formation of clumps when the medium was compressed by hand (Day et al., 2022). Each medium was weighed at 20 g and placed into a Petri dish (Uruilal et al., 2012). The media were then sterilised in an autoclave at 121 °C for 60 minutes at 1 atm. After cooling, the media were inoculated

with *Trichoderma* spp. by placing one 5-mm agar plug containing the fungal isolate at the centre of the medium surface.

Growth of Trichoderma spp. on Propagation Medium

The observed parameters included fungal growth, assessed using the average incubation period and the difference in medium weight before and after incubation. The average incubation period was determined for all treatment combinations by recording the day on which fungal growth was first observed on the propagation media, calculated from the time of inoculation (Gusnawaty et al., 2017). The difference in medium weight before and after incubation was measured by weighing the medium before inoculation and after incubation, when the entire medium surface had been fully colonised by *Trichoderma* spp., using a digital balance. These measurements were conducted for all treatment combinations.

Conidial Quality Test of Trichoderma spp.

The quality of *Trichoderma* spp. conidia produced on the propagation media were evaluated based on conidial density and viability. Samples were randomly collected from each fungal culture grown on each type of propagation medium. The conidial assessment followed the method established by BBPOPT (2023). For conidial density determination, each sample was homogenised by shaking or stirring. One gram of each sample was weighed and placed into a sterilised 100 mL Erlenmeyer flask. Distilled water was added to reach a volume of 100 mL, followed by the addition of one drop of Tween 80. The flask was covered with aluminium foil, and the conidial suspension was homogenised using a vortex mixer for 15 minutes, resulting in a 10^{-2} dilution. A *Neubauer Improved hemocytometer* was placed on the microscope stage and covered with a cover glass. The counting chamber was located at 100× magnification, and the 0.2 mL aliquot of the 10^{2} diluted suspension was then observed at 400× magnification. A 0.2 mL aliquot of the 10^{-2} diluted suspension was taken using a micropipette and introduced into both the upper and lower channels of the hemocytometer (Figure 1).

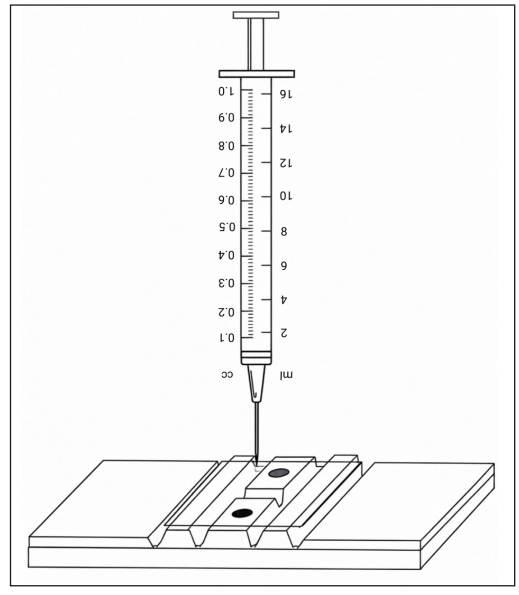


Figure 1. Application of conidial suspension onto the counting chamber
*Source: Center for Forecasting of Plant Pest Organisms (BBPOPT) (2023)

The suspension was allowed to stand for one minute to stabilise the conidia. Conidia were counted diagonally in squares a, b, c, d, and e on both counting chambers using a hand counter. Only conidia within the counting squares and those touching the left and upper boundary lines were counted (Figure 2).

After obtaining the conidial count from both the upper and lower counting chambers, the number of conidia per millilitre was calculated using Equation 1.

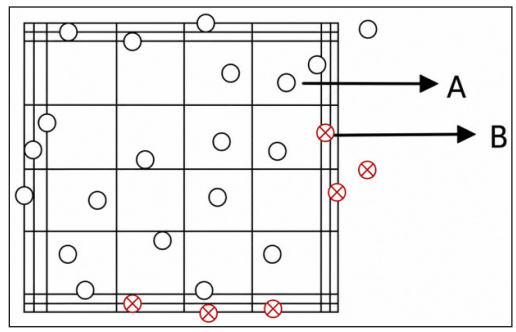


Figure 2. Counting of fungal conidia: (A) conidia included in the count and (B) conidia excluded from the count
*Source: Center for Forecasting of Plant Pest Organisms (BBPOPT) (2023)

$$S = \frac{\bar{X}}{L \times t \times d} \times 10^3 \qquad [1]$$

Where:

S : Conidial density (conidia/mL)
$\bar{X}$: Average number of conidia counted in the upper and lower chambers
L : Counting area 0.04 mm²
T : Chamber depth (0.1 mm)
D : Dilution factor
10^3 : Volume conversion factor (1 ml = 10^3 mm^3)

Conidial viability testing was conducted as a continuation of the conidial density assessment. PDA medium was prepared in Petri dishes and cut into agar discs (0.5 cm diameter) using a cork borer. Three agar discs were placed on a sterile microscope slide as replicates. The 10^{-2} conidial suspension was re-homogenised using a vortex mixer for 3 minutes and subsequently applied to each agar disc using a 1 mL syringe. Each disc received one drop of the suspension and was covered with a cover glass. The prepared slides were placed inside Petri dishes supported by two toothpicks, and two rolls of moist cotton were added to maintain humidity. The Petri dishes were sealed with cling wrap and incubated in the dark. Conidial viability was observed after 16 hours of incubation using a microscope at 400× magnification. Conidia were considered germinated when the germ tube length reached at least half the length of the conidium (Sinclair & Dhingra, 1995). Viability was calculated using Equation 2.

$$\text{Viability} = \frac{\Sigma \text{ Germinated conidia}}{\text{Total observed conidia}} \text{ x } 100\% \qquad [2]$$

Test of Two Trichoderma *spp. Isolates Against Downy Mildew Incidence in Maize*

This test was conducted as a continuation of the conidial quality assessment. Five treatments were applied: control (A), BBPOPT isolate on maize medium (B), BBPOPT isolate on rice bran medium (C), BBPPTP isolate on maize medium (D), and BBPPTP isolate on rice bran medium (E). The maize seeds were prepared by soaking them in water for 24 hours, then draining and incubating in plastic boxes lined with moist tissue paper for another 24 hours. Seed treatment with *Trichoderma* spp. was performed by preparing a 50 mL conidial suspension with a known density and adding 12.5 g of granulated sugar (Turnip, 2015). The conidial suspensions used for seed treatment were prepared based on the measured conidial density from each treatment. Equal suspension volumes were applied to seeds to maintain consistency among treatments. The maize seeds were soaked in the suspension for 30 minutes, while the control seeds were soaked in water containing the same concentration of sugar. Following treatment, the seeds were then drained and placed in plastic boxes according to treatment codes, lined and covered with moist tissue paper to stimulate germination. The seeds were incubated for an additional 48 hours before inoculation with downy mildew.

Downy mildew inoculation was performed using the leaf-insertion method (Adhi et al., 2019). Each treated seed, including the control, was inoculated by inserting maize leaf tissue infected with *Peronosclerospora* spp. into the seed. The inoculated seeds were incubated for 24 hours and subsequently planted at a density of eight seeds per polybag. Observations were conducted from one to five weeks after planting to assess downy mildew incidence. Disease incidence was calculated using Equation 3 (Adhi et al., 2019).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \text{ x } 100\% \qquad [3]$$

The Area Under the Disease Progress Curve (AUDPC) is a single value that describes disease development and is derived from integrating multiple observations of disease intensity, disease incidence, or both (Simko & Piepho, 2012). AUDPC was calculated using Equation 4 (Landeo, 1999, in Kurniawan et al., 2018).

$$AUDPC = \sum_{i=1}^{n}(X_{t+1} + X_t)(D_{t+1} - D_t) \qquad [4]$$

Where:

X_t : Percentage of downy mildew incidence at time t

X_{t+1} : Percentage of downy mildew incidence at the subsequent observation time $t + 1$

$(D_{t+1} - D_t)$: Observation interval between two consecutive assessments

Data were further analysed using Duncan's Multiple Range Test (DMRT) at the 5% significance level to compare treatment means.

RESULTS AND DISCUSSIONS

Growth of Two *Trichoderma* spp. Isolates on Different Propagation Medium

Incubation Period

Both *Trichoderma* spp. isolates showed visible growth on both propagation media at 2 days after inoculation (DAI) (Table 1). Fungal growth was characterised by the emergence of hyphae spreading from the inoculation point toward the surface of the propagation medium. Both isolates completely colonised the surface by 10 DAI; in contrast, on rice bran medium, full colonisation occurred between 10 and 18 DAI (Figure 3).

Table 1
Average incubation period of two Trichoderma *spp. isolates on different propagation Media (Days)*

Treatment	Incubation Period (Days)
t_1m_1	2
t_1m_2	2
t_2m_1	2
t_2m_2	2

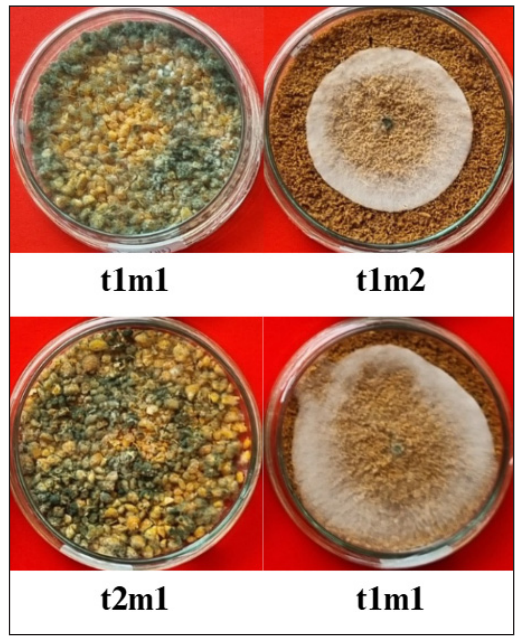


Figure 3. Growth of two *Trichoderma* spp. isolates on different propagation media at 10 Days after Inoculation (DAI)
*Source: Personal documentation (Ida Nurhelawati, 2025)

Based on the average incubation period, both isolates began to grow at 2 DAI, indicating that both propagation media supported *Trichoderma* spp. growth. However, fungal growth was faster on maize medium compared to rice bran medium. Complete colonisation occurred uniformly at 10 DAI on maize medium, whereas colonisation on rice bran medium was more variable. These differences are likely attributable to variations in nutrient composition between the two media. Fungal growth is strongly influenced by the availability of essential nutrients such as carbohydrates and proteins (Basu et al., 2015). Maize contains a higher carbohydrate content, reaching up to 75.64% (Lalujan et al., 2017), whereas rice bran contains approximately 34-62% carbohydrates (Chakraborty & Budhwar, 2018). The higher carbohydrate content in maize likely contributed to the faster mycelial growth of *Trichoderma* spp. observed in this study.

Difference in Medium Weight before and After Inoculation

The difference in medium weight was determined by subtracting the weight before inoculation from the weight after fungal colonisation. Statistical analysis indicated no significant interaction between fungal isolates and propagation media on the difference in medium weight. However, a significant main effect of propagation medium was observed (Table 2). The results indicated that rice bran medium (m_2) exhibited the greatest reduction in medium weight (1.20 g), compared with maize medium (0.88 g).

Table 2
Effect of two Trichoderma *spp. isolates grown on different propagation media on the difference in medium weight before and after inoculation*

Treatment	Weight Before Inoculation (g)	Weight After Inoculation (g)	Weight Difference (g)
Fungal Isolate			
t_1	20	18.75	1.03 a
t_2	20	18.94	1.06 a
Propagation Medium			
m_1	20	19.12	0.88 a
m_2	20	18.80	1.20 b

Note. Different letters indicate a significant difference based on Duncan's Multiple Range Test (DMRT) at the 5% significance level

The greatest reduction in medium weight was observed in the rice bran treatment, with a value of 1.20 g. This finding is consistent with those of Gusnawaty et al. (2017), who also observed the greatest weight reduction in the rice bran medium. The difference in medium weight before and after incubation reflects the metabolic activity of *Trichoderma* spp. The greater weight loss observed in the rice bran medium suggests that its nutrient composition is well suited to the nutritional requirements of *Trichoderma* spp., enabling more rapid substrate decomposition (Gusnawaty et al., 2017).

Rice bran contains major carbohydrate components, including hemicellulose (8.7-11.4%), cellulose (9-12.8%), starch (5-15%), and β-glucan (1%) (Astawan & Febrinda, 2010). *Trichoderma* spp. degrades substrates such as starch, pectin, and cellulose by producing extracellular enzymes, including amylase, pectinase, cellulase, and chitinase, which facilitate nutrient utilisation for growth (Yparraguirre & Galliani-Pinillos, 2020). The high reduction in medium weight therefore further indicates that *Trichoderma* spp. have strong potential as effective organic matter decomposers.

Conidial Quality of Two *Trichoderma* spp. Isolates on Different Propagation Media

Conidial Density

Statistical analysis showed no interaction and no significant main effects of fungal isolates or propagation media on conidial density (Table 3). All treatments produced similar conidial

densities, averaging 10^8 conidia mL^{-1}. The highest conidial density was observed in the BBPPTP isolate (t_2), with 2.55×10^8 conidia mL^{-1}. However, this value was not significantly different from t_1 (2.25×10^8 conidia mL^{-1}), maize medium (m_1), or rice bran medium (m_2), both of which produced 2.40×10^8 conidia mL^{-1}. These results indicate that both propagation media supported the sporulation of *Trichoderma* spp. effectively.

Table 3
Effect of two Trichoderma *spp. isolates grown on different propagation media on conidial density (10^8 conidia mL^{-1})*

Treatment	Conidial Density (10^8 conidia mL^{-1})
Fungal isolate	
t_1	2.25 a
t_2	2.55 a
Propagation medium	
m_1	2.40 a
m_2	2.40 a

Note. Same letters indicate non-significant differences based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

The assessment of conidial density for two *Trichoderma* spp. isolates grown on different propagation media resulted in an average density of 10^8 conidia mL^{-1}. These findings indicate that both isolates produced comparable amounts of conidia across the two types of propagation media tested. Furthermore, the conidial densities obtained in this study exceeded the minimum standard established by the Badan Standardisasi Nasional (2014), which requires $\geq 10^6$ conidia mL^{-1}. High conidial density is closely associated with *Trichoderma* spp.'s ability to suppress pathogen development. This is consistent with the findings of Nabila *et al*. (2021), who reported that a conidial density of 10^7 conidia mL^{-1} was capable of suppressing Fusarium wilt by more than 50%.

Conidial Viability

Conidial viability of *Trichoderma* spp. was observed microscopically after 16 hours of incubation. Conidia were considered germinated when the germ tube length reached at least half the length of the conidium (Sinclair & Dhingra, 1995) (Figure 4).

Statistical analysis revealed a significant interaction between fungal isolates and propagation media on conidial viability (Table 4). The highest conidial viability was observed in the BBPOPT isolate propagated on maize medium (t_1m_1), at 98.61%, and was not significantly different from that

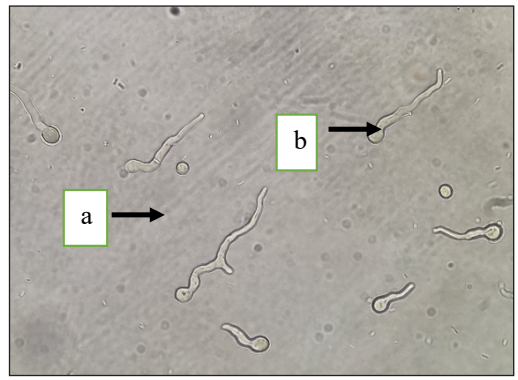


Figure 4. Conidia of *Trichoderma* spp.: (a) Germinated conidia and (b) Non-germinated conidia
*Source: Personal documentation (Ida Nurhelawati, 2025)

Table 4
Effect of two Trichoderma *spp. isolates grown on different propagation media on conidial viability (%)*

Factor t	Factor m	
	m_1	m_2
t_1	98.61 a B	82.70 a A
t_2	96.47 a B	91.46 b A

Note. Different letters indicate significant differences based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

of the BBPPTP isolate on maize medium (t_2m_1), at 96.47%. However, both treatments differed significantly from t_1m_2 and t_2m_2. The lowest conidial viability was recorded for the BBPOPT isolate propagated on rice bran medium (t_1m_2), at 82.70%.

Conidial viability reflects the ability of conidia to germinate and grow normally under optimal conditions (Utami et al., 2023). In this study, an interaction was observed between two *Trichoderma* spp. isolates and different propagation media on fungal conidial viability. The highest viability was recorded in treatments t_1m_1 and t_2m_1, reaching 98.61% and 96.47%, respectively. These values exceed the minimum conidial viability standard for *Trichoderma* spp., set at≥ 60% by the Badan Standardisasi Nasional (2014). The corn-based medium had a significant effect on the conidial viability of both *Trichoderma* spp. isolates. According to Utami et al. (2023), conidial viability is influenced by several factors, including culture age, nutrient content of the growth medium, and environmental factors such as light, temperature, and moisture. Nutrients present in the propagation media can affect fungal survival, sporulation, and antagonistic ability (Mufida et al., 2023). Furthermore, high conidial viability is crucial for supporting fungal growth and development as a biological control agent (Nurbailis & Martinius, 2011).

Effect of Two *Trichoderma* spp. Isolates on Downy Mildew Incidence in Maize

Incidence of Downy Mildew Disease in Maize Plants

Downy mildew disease was first observed one week after planting (WAP). Disease symptoms appeared on newly emerging leaves, characterised by elongated chlorotic streaks parallel to the leaf veins (Figure 5a). In the early morning, white conidial masses were observed on the abaxial surface of infected leaves. Microscopic observations revealed that the conidiophores of the downy mildew pathogen exhibited three levels of branching, were equipped with sterigmata bearing terminal conidia, and had a spherical to sub-spherical shape.

At one week after planting (WAP), the highest percentage of downy mildew incidence was observed in the control treatment (3.93%) and based on statistical analysis, was not

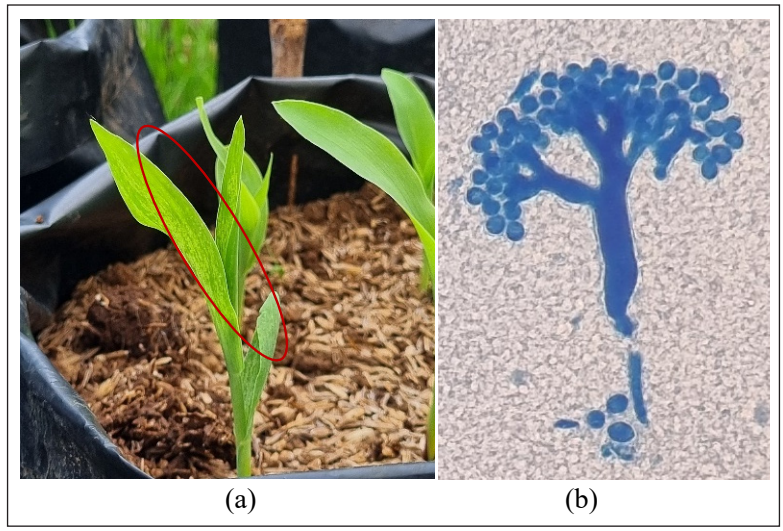


Figure 5. Downy mildew disease: (a) Symptoms of downy mildew at 1 week after planting (WAP) and (b) Conidiophores and conidia of *Peronosclerospora* spp
*Source: Personal documentation (Ida Nurhelawati, 2025)

significantly different from that in treatment t_1m_2 (1.43%) (Table 5). However, disease incidence in the control treatment (3.93%) was significantly higher than in treatments t_1m_1, t_2m_1, and t_2m_2, which showed no symptoms of downy mildew infection (0%). At the second WAP, downy mildew symptoms were detected in all treatments; however, the percentage of disease incidence was significantly lower than in the control. At 3, 4, and 5 WAP, the percentage of disease incidence in the control was not significantly different from that of the other treatments, although the control consistently showed higher disease incidence. At the final observation (5 WAP), downy mildew incidence in the control treatment reached 13.04%, while the lowest disease incidence was recorded in treatment t_2m_1.

Table 5
Effect of two fungal isolates grown on different propagation media on downy mildew incidence (%)

Treatment	Downy Mildew Incidence (%)				
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP
A (control)	3.93 b	6.61 b	7.86 a	9.11 a	13.04 a
B (t_1m_1)	0.00 a	2.68 a	5.28 a	6.51 a	7.76 a
C (t_1m_2)	1.43 ab	2.68 a	5.18 a	7.68 a	10.36 a
D (t_2m_1)	0.00 a	1.22 a	5.00 a	7.50 a	7.50 a
E (t_2m_2)	0.00 a	2.58 a	5.17 a	7.75 a	7.75 a

Note. Values followed by different letters indicate significant differences according to Duncan's Multiple Range Test at the 5% significance level

Based on the experimental results, the lowest percentage of downy mildew incidence was observed at 1 week after planting (WAP), during which disease symptoms were detected only in the control and treatment t_1m_2. At 2 WAP, all treatments exhibited symptoms of downy mildew; however, the percentage of disease incidence in all treated groups remained lower than that in the control. At 3, 4, and 5 WAP, no significant differences in disease incidence were observed among treatments. These results indicate that the two *Trichoderma* spp. isolates propagated on different media suppressed downy mildew incidence only during the early growth stage of maize, specifically within 1-2 WAP. This early suppression may be attributed to *Trichoderma* spp.'s ability to delay the pathogen's incubation period during the host plants' initial growth phase. The relatively high disease incidence observed at 3, 4, and 5 WAP may be influenced by environmental conditions that favour the development and spread of downy mildew. This observation is consistent with the findings of Purwanto et al. (2016), who reported that environmental factors play a critical role in the dissemination of downy mildew disease. In the present study, the temperature during the observation period ranged from 24.5 °C to 29 °C, with relative humidity between 98% and 99%. The optimum temperature for downy mildew development is below 24 °C with relative humidity around 90% (Semangun, 2008). Although the temperature conditions during the experiment were not ideal for downy mildew development, the high relative humidity and continuous availability of susceptible host plants supported pathogen establishment and disease development. Therefore, under environmental conditions conducive to disease development, the treatments applied in this study were insufficient to suppress downy mildew incidence at later growth stages effectively.

Area Under the Disease Progress Curve (AUDPC)

Statistical analysis showed that AUDPC values among all treatments were not significantly different (Table 6). The highest mean AUDPC value was observed in the control treatment (224.35), while the lowest was recorded in treatment t_2m_1 (122.50). The highest downy

Table 6
AUDPC values and percentage of downy mildew suppression

Treatment	AUDPC					Means AUDPC	Inhibition (%)
	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP		
A (control)	196.88	224.95	174.93	218.75	306.25	224.35 a	0.00
B (t_1m_1)	0.00	175.00	116.73	175.00	174.93	128.33 a	42.80
C (t_1m_2)	175.00	218.75	131.25	0.00	224.95	149.99 a	33.15
D (t_2m_1)	109.38	109.38	0.00	175.00	218.75	122.50 a	45.40
E (t_2m_2)	109.38	186.66	0.00	163.42	218.75	135.64 a	39.54

Note. Values followed by different letters indicate significant differences according to Duncan's Multiple Range Test at the 5% significance level

mildew suppression was observed in treatment t_2m_1 (45.40%), whereas the control treatment showed no suppression (0%).

The results of the evaluation of two *Trichoderma* spp. isolates propagated on different media indicated no significant differences in AUDPC values compared with the control. The highest AUDPC value was observed in the control treatment (224.35), while the lowest was recorded in treatment t_2m_1 (122.50). A higher AUDPC value indicates greater disease development, whereas a lower AUDPC value indicates reduced disease progression (Suganda, 2001 in Kurniawan et al., 2018). The AUDPC value was inversely related to the percentage of disease suppression; lower AUDPC values were associated with higher levels of disease suppression. The highest suppression of downy mildew was observed in treatment t_2m_1 (45.40%).

CONCLUSION

The propagation medium had a significant independent effect on the reduction in medium weight before and after inoculation with *Trichoderma* spp. Using rice bran media reduces medium weight by up to 1.20 g.

The combination of two *Trichoderma* spp. isolates and different propagation media had no significant effect on conidial density, which ranged from 2.25×10^8 to 2.55×10^8 conidia mL^{-1}. However, it had a significant effect on conidial viability. The highest conidial viability was recorded in *Trichoderma* spp. isolates originating from BBPOPT and BBPPTP, propagated on maize medium, with viability values of 98.61% and 96.47%, respectively.

The BBPOPT and BBPPTP isolates on maize medium showed the lowest numerical AUDPC value (128.33 and 122.50) and the highest numerical disease suppression (42.8% and 45.40%). However, the differences among treatments were not statistically significant. It indicates that the Trichoderma spp. treatments delayed disease development during the early growth stage but did not significantly suppress overall downy mildew progression.

IMPLICATIONS OF THE STUDY

This study demonstrates that using local agricultural by-products, such as cracked maize, for the mass production of *Trichoderma* spp. offers a highly viable, eco-friendly alternative to synthetic fungicides in maize farming. These findings provide a low-cost, sustainable framework that empowers resource-limited farmers to produce effective biocontrol agents, supporting green agriculture initiatives.

The implications of this study are expected to show that *Trichoderma* spp. isolates propagated on corn media, both originating from BBPOPT and BBPPTP, have potential as biological control candidates due to their ability to produce high-quality inoculum. However, further validation through multi-location and multi-season field experiments is required before recommending their practical application for downy mildew management in maize production.

LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

This study demonstrated the potential of *Trichoderma* spp. propagated on different substrates to improve conidial quality and suppress downy mildew development in maize. However, several limitations should be acknowledged. First, the experiment was conducted under a single agroecological condition and within a single planting season, which may limit the broader applicability of the findings across different environmental conditions, cropping systems, and pathogen pressures. The efficacy of biological control agents is strongly influenced by environmental factors, including temperature, humidity, soil physicochemical properties, and indigenous microbial communities, all of which can affect the establishment and antagonistic performance of *Trichoderma* spp. under field conditions. Second, the study evaluated disease suppression primarily through disease incidence and AUDPC parameters without further investigation of physiological, biochemical, or molecular plant responses associated with induced resistance mechanisms. Consequently, specific pathways underlying antagonistic interactions between *Trichoderma* spp. and *Peronosclerospora* spp. remain insufficiently understood. Third, although conidial density and viability were assessed, additional indicators of inoculum quality, including shelf life, rhizosphere colonisation ability, enzymatic activity, secondary metabolite production, and persistence under field conditions, were not examined. Furthermore, the study used only two isolates and two propagation substrates, which may not fully represent the diversity of Trichoderma strains or the locally available organic substrates with potential for large-scale bioformulation development.

Future studies should therefore focus on multi-location, multi-season field evaluations to determine the consistency and stability of *Trichoderma*-based biological control across diverse environmental conditions. Further investigations integrating molecular, transcriptomic, and metabolomic approaches are also required to elucidate the mechanisms underlying induced systemic resistance, pathogen inhibition, and plant-microbe interactions. In addition, future research should explore optimisation of carrier formulations, shelf-life stability, and compatibility with other integrated disease management strategies to enhance field efficacy and commercialisation potential. Screening additional indigenous *Trichoderma* isolates and low-cost agricultural by-products as propagation substrates may also provide opportunities to develop more adaptive, economically feasible, and environmentally sustainable biocontrol technologies for maize production systems.

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Allometric Biomass Equations and Form Factor Determination for Four-Year-Old *Eucalyptus* Hybrid DH3229

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ABSTRACT

Effective inventory methods are crucial for managing short-rotation plantations. This study evaluates the aboveground biomass and morphometric characteristics of four-year-old *Eucalyptus* hybrid DH3229. We used a stratified random sampling method to destructively sample 30 trees from a low-maintenance terrain plot. Each sample was fractionated and weighed across fresh, air-dried, and oven-dried states. Results demonstrated that total biomass, particularly for the fresh weight equation, exhibited greater performance across all log positions compared to air-dry weight and oven-dry weight equations. Using Newton's formula as a volumetric benchmark, error analyses (Mean Bias Error, Root Mean Square Error, and Standard Deviation) confirmed the reliability of Smalian's, Huber's, and Bruce's formulas, with Huber's method consistently exhibiting the lowest error across all evaluated metrics and log intervals. An estimated empirical form factor was determined to be 0.579, surpassing the previously reported values for older age classes (6-9 years). These findings provide reliable, site-specific allometric equations and form factors for early-age short-rotation plantations.

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INTRODUCTION

Malaysia's hardwood resources have struggled to meet the increasing demand from the domestic wood processing sector. To reduce reliance on natural forests,

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extensive forest plantations are aggressively commercialised. Efforts to optimise wood production frequently prioritise species traits such as rapid growth, high timber yield, and pest resistance (Climent et al., 2024). *Eucalyptus* species are preferentially selected for reforestation due to their adaptability to tropical and subtropical climates, resilience to moisture stress, nutrient-poor soils, fire, and pests (Cookson & McCarthy, 2013; Ginwal, 2014). With the expanding interest in these species, a thorough understanding of their resource characteristics and end-use potential has become essential.

The harvest cycle for fast-growing species for solid timber production requires a period of six to nine years to reach optimal wood density (Hii et al., 2017; Taziwa, 2023). However, the growth pattern of *Eucalyptus* species shows that the Mean Annual Increment (MAI) peaks at three years of age and declines significantly in subsequent years (Harwood & Nambiar, 2014; Hung et al., 2015). This suggests the potential to meet the demand of medium-value product industries, such as packaging materials and finger-jointed laminated plywood, as early as four years of age.

Under tropical conditions (mean annual temperature: 26 °C; rainfall: 2500 mm), *Eucalyptus* hybrid (*E. urophylla* × *E. grandis*) exhibits 23% greater DBH $p< 0.05$ and an 18% increase in height (Yahya, 2020). Meanwhile, the survival rate was high (> 90%) compared to *E. pellita* (Zuhaidi et al., 2020). Productivity data from Sabah Softwood Berhad (5° N, 118° E) corroborated these findings, in which *E.* hybrid achieved 44% higher mean annual increment (39.7 versus 27.6 $m^3\ ha^{-1}\ yr^{-1}$ over a nine-year assessment period) (Japarudin et al., 2020). Despite extensive research on *Eucalyptus* at steady harvest ages (six to nine years), destructive biomass and volumetric data for early-stage tropical plantations remain limited.

Estimating tree aboveground biomass involves the allometric relationship between the biomass of whole trees (dependent variable), as well as readily measured parameters such as the diameter at breast height (DBH) and stem height (H), which serve as independent variables. Developing allometric equations with destructive biomass of a small number of trees by considering small and large tree sizes was preferable instead of mean-tree methods that require the harvesting of all planted trees in developing the equation (Chave et al., 2014; Fayolle et al., 2013; Komiyama et al., 2008).

Good precision of tree variables at the plot level can also be used to estimate individual tree volume by applying mathematical expressions, including Newton, Smalian, Huber and Bruce (Bruce, 1982; Husch et al., 2003). To overcome the overestimation or underestimation of volume estimation, a stem taper model is required in predicting changes in the volume in stem form from the ground to the tip, and a form factor that adjusts the volume of a cylinder to match the actual tree form (Silva et al., 1994; Tenzin et al., 2017).

While traditional biomass determination incorporates multiple secondary variables such as total height and crown distribution, these measurements are often time-consuming, expensive, and less critical for uniform, even-aged monoculture plantations. To maximise

operational efficiency, this study used the DBH parameter as the sole predictive variable. The specific objectives of this study were to: (1) develop species-specific, DBH-driven allometric equations for aboveground biomass components, and (2) preliminarily determine the form factor and evaluate the accuracy of the standard volume formula.

MATERIALS AND METHODS

Study Site

Four-year-old *E. hybrid* DH3229 trees were selected randomly from a plantation at Mustafa Bubjee College (2.9783° N, 101.7156° E, 54 m a.s.l), planted at a spacing of 1.0 m × 1.5 m. The plot was established on sloped terrain (22.4°) within a university campus and maintained under low-intensity silvicultural conditions. The site received annual rainfall (2,000 - 2,500 mm) without artificial irrigation. Weed interference was managed with regular manual slashing, and no intensive routine of commercial fertilisation was applied post-establishment, closely reflecting the actual smallholder agroforestry and degraded land rehabilitation practices.

Field Sampling and Laboratory Preparation

Destructive sampling procedures were adopted from Doruska et al. (2006), Abdul-Hamid et al. (2022), and Hossain et al. (2023). Tree diameter was measured at DBH approximately 1.3 m above the ground using the Forestry Suppliers Metric Fabric Diameter Tape (Model 283D/10M, Jackson, MS, USA). To guarantee that the samples accurately reflected the stand's distribution, a stratified random sampling approach was used to select 30 standing trees (DBH: 8.10 cm to 12.50 cm). In uniform, even-aged plantation settings, a destructive sample size of 30 trees is statistically robust for establishing preliminary species-specific allometric baselines and form factors. To evaluate potential biological shifts in biomass allocation as trees surpass the stand's mean diameter (~10 cm), the harvested samples were deliberately stratified into two equal classes: DBH ≤10 cm (n=15) and DBH >10 cm (n=15).

The fresh weight of each component (stems, leaves, and branches) from the 30 destructive trees was measured in situ using a digital scale (Model 150 kg/ 200 kg Proace Electronic Scale) (Figure 1). For all trunk sample trees, the trunk diameter was measured at the lower, middle, and upper part of the log that was cut from ground level in the following lengths: 0-0.3 m, 0.3-1.3 m, 3.3-5.3 m and then every 2 m length. Thereafter, 500 g of the fresh weight of each part was taken to the laboratory. To determine air-dry weight (ADW), the samples were stored in a well-ventilated, shaded room at ambient tropical temperatures and weighed periodically until equilibrium moisture content was reached (constant weight). Subsequently, to determine the absolute oven-dry weight (ODW), the samples were dried in a laboratory oven at 70 °C for 15 days until a strict constant weight was attained, ensuring complete moisture removal.



Figure 1. The diameter and fresh weight at the specified trunk length were collected, and samples were cut into a disc shape, approximately 2 to 3 cm thick and weighed *in situ*

Biomass Estimation

The biomass of each component was calculated by (Eq. 1):

$$B = M_{Fre} \times \left(\frac{M_{sam,Dry}}{M_{sam,Fre}}\right) \quad [1]$$

where M_{Fre} is the fresh mass of each component, and $M_{sam,\ Dry}$ and $M_{sam,\ Fre}$ are the dry mass and fresh mass of the samples of each component, respectively. The total aboveground biomass was obtained by summing the biomasses of each component.

Volume and Form Factor Estimation

The observed tree volume was calculated using Newton's formula (Eq. 2) (Husch et al., 2003);

$$V_{i} = \left(\frac{1}{6}\right) \times f \times L \times (d^2{}_A + 4d^2{}_M + d^2{}_B) \quad [2]$$

where;
V_i = Log volume at $_i$th (m^3)
L = Length of log (m)
f = 0.00007854 (metric units)
d_A = Diameter at the small log end (cm)
d_B = Diameter at the large log end (cm)
d_M = Diameter at the mid-length log end (cm)

Meanwhile, for predicted volume, various methods have been explored in estimating log volume, with log formulas being among the most used due to their straightforward application with available datasets that include one or two measurements of log diameter (Mushar et al., 2020). We selected three commonly used volume formulas to obtain the necessary coefficients for volume calculations, ensuring accuracy and relevance to our study. The statistical analyses were performed using SPSS. We employed nonlinear regression models at the 5% significance level to compute each equation. For our study, we specifically considered Smalian's (Husch et al., 2003), Huber's (Husch et al., 2003), and Bruce's formula (Bruce, 1982) (Table 1).

Table 1
Predicted volume estimation by using Smalian's, Huber's and Bruce's formulas by using a statistical method for different log sectional lengths

Volume Formula	Equations	
Smalian	$V_i = \left(\frac{1}{2}\right) \times L \times (d^2{}_A + d^2{}_B)$	[3]
Huber	$V_i = f \times L \times (d^2{}_M)$	[4]
Bruce	$V_i = f \times L \times [0.25\ (d^2{}_B) + 0.75\ (d^2{}_A)]$	[5]

Note. V_i = log volume at i^{th} (m^3), L = length of log (m), f = 0.00007854 (metric units), d_A = diameter at the small log end (cm), d_B = diameter at the large log end (cm), and d_M = diameter at the mid-length log end (cm)

The observed tree volumes calculated using Newton's formula were then applied to the real form factor. We calculated the real form factor value for each tree sample using the given formula in Eqs. 6 and 7:

$$FF = \frac{V_{sum\ of\ logs}}{BA * Total\ Height} \quad [6]$$

$$BA = \frac{\pi \times DBH^2}{40{,}000} \quad [7]$$

The volume (V) of the tree is measured in m^3, calculated using Equation (1), where BA is the basal area of the tree (DBH) measured in cm, H is the total tree height measured in meters (m), and FF represents the form factors of a tree. 40,000 for denominators was used to convert the DBH from cm to square meters for the basal area, which can be summarised as $0.00007854 \times DBH^2$.

Accuracy Assessment

The volume estimates derived from Smalian's, Huber's, and Bruce's formulas were evaluated in comparison to the reference tree volumes calculated using Newton's formula. To avoid a logical fallacy, Newton's formula was strictly employed as the benchmark "observed" volume and was not evaluated for predictive error. Meanwhile, the three predictive formulas' performance was assessed using three standard statistical metrics: Mean Bias Error (MBE), Root Mean Square Error (RMSE), and Standard Deviation (SD).

The Mean Bias Error (MBE) estimates the average systematic error between the predicted and observed values. A positive MBE indicates an overestimation, while a negative MBE indicates an underestimation. It is calculated using Eq. 8:

$$MBE = \frac{1}{n}\sum_{i=1}^{n}\left(V_{pred,i} - V_{obs,i}\right) \quad [8]$$

The Root Mean Square Error (RMSE) evaluates the overall predictive accuracy by measuring the magnitude of the errors. Lower RMSE values indicate improved model performance. It is calculated using Eq. 9;

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(V_{pred,i} - V_{obs,i}\right)^2} \quad [9]$$

At the end of the accuracy assessment, the Standard Deviation (SD) of the errors was calculated to assess the precision of the models, indicating how widely the prediction errors are dispersed around the mean error. It is calculated using Eq. 10;

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(E_i - MBE)^2}{n-1}} \quad [10]$$

where $V_{pred,i}$ is the predicted log volume utilising Smalian's, Huber's or Bruce's formulas (m^3), $V_{obs,i}$ is the observed log volume utilising Newton's formula (m^3), E_i is the individual estimation error ($V_{pred,i} - V_{obs,i}$) and n is the total number of log samples.

Statistical Analysis

For the differences in biomass accumulation between the two size classes (DBH ≤10 cm and DBH >10 cm), an Independent Samples T-Test was conducted using IBM SPSS Statistics software (version 25.0; IBM Corp., Armonk, NY, USA). The relationships of three independent and dependent variables (DBH, total height, and biomass components)

were examined using Pearson's correlation analysis. Further, non-linear regression models were projected at a 5% significance level to obtain the species-specific allometric equations ($Y = aD\hat{}b$) and volume estimation coefficients. Significance levels were set at $p < 0.05$ for statistically significant differences and $p < 0.001$ for highly significant differences.

RESULTS

Overview of the Destructive Samples

The *E.* hybrid DH3229 trees sampled in this study presented a wide range of characteristics. The average DBH was 10.07 cm, with values ranging from a minimum of 8.10 cm to a maximum of 12.50 cm. Similarly, the average tree height was 12.13 m, with the smallest tree measuring 8.80 m and the tallest 15.10 m (Table 2). Figure 2 illustrates the relationship between DBH and total tree height for the 30 sampled *E.* hybrid DH3229 trees, which were assessed for aboveground biomass, real tree volume, predicted volume and form factor determination. The coefficient of determination ($R^2 = 0.097$) indicates a weak correlation between DBH and tree height, suggesting high variability in height growth within this DBH range.

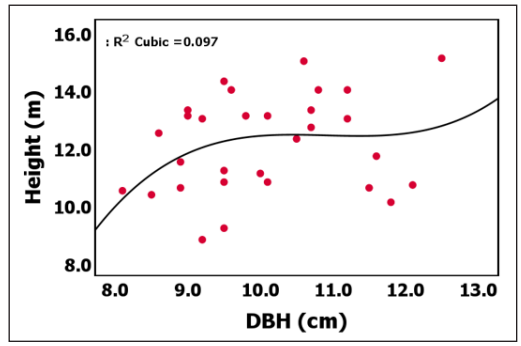


Figure 2. The relationship of tree height and tree diameter at breast height (DBH) for the 30 sampled trees

Table 2
Characteristics of 30 destructive sample trees of four years of Eucalyptus hybrid DH3229

Variable	Sum	Mean	Min	Median	Max	Std.
No. of tree	30	-	-	-	-	-
Height (m)	-	12.13	8.80	12.40	15.10	1.683
DBH (cm)	-	10.07	8.10	9.90	12.50	1.150
Total Volume (m^3)	1.751	0.058	0.030	0.056	0.101	0.018

Biomass Estimation

The independent-sample *t*-test revealed substantial mean differences in biomass accumulation between the two diameter classes (≤10 cm versus >10 cm) (Table 3). For instance, for oven-dry weight of full height (ODWFH), the larger tree class yielded a mean biomass of 35.32 ± 2.51 kg compared to the smaller class averaging only 19.97 ± 1.34 kg. This represents an absolute mean difference of 15.35 kg of solid dry wood per tree. This

allocation pattern stays consistent for the fresh weight of full height (FWFH), where the larger diameter class achieved a mean weight of 67.48 ± 3.00 kg, compared to 44.10 ± 2.32 kg in the smaller class, resulting in a mean difference of 23.38 kg.

Table 3
Independence t-test of tree at DBH ≤ 10 cm and DBH >10 cm for fresh weight, air-dry weight and oven-dry weight states

Components	Mean ± SD (≤ 10 cm, *n* =15)	Mean ± SD (> 10 cm, *n* = 15)	Mean Diff.	*t*	*df*	*p*
FH (m)	11.75 ± 1.71	12.50 ± 1.62	0.75	-1.23	28	0.230
DBH (cm)	9.12 ± 0.47	11.03 ± 0.76	1.91	-8.28	23.42	< 0.001
FWFH (kg)	44.10 ± 9.02	67.48 ± 11.64	23.38	-5.08	28	< 0.001
ADWFH (kg)	23.30 ± 5.79	40.56 ± 11.81	17.26	-5.38	20.36	< 0.001
ODWFH (kg)	19.97 ± 5.21	35.32 ± 9.73	15.35	-6.12	21.40	< 0.001
FWMH (kg)	42.23 ± 8.94	64.69 ± 11.06	22.46	-5.00	28	< 0.001
ADWMH (kg)	21.41 ± 6.07	38.46 ± 11.73	17.05	-5.07	21	< 0.001
ODWMH (kg)	18.74 ± 5.38	33.47 ± 9.9	14.73	-2.13	21.60	< 0.001
FWL (kg)	3.86 ± 0.86	4.62 ± 1.09	0.76	-3.9	28	0.040
ADWL (kg)	1.98 ± 0.53	2.96 ± 0.81	0.98	-3.48	28	< 0.001
ODWL (kg)	1.73 ± 0.55	2.67 ± 0.89	0.94	-3.27	28	< 0.001
FWB (kg)	6.04 ± 1.94	9.72 ± 3.9	3.68	-2.27	28	0.030
ADWB (kg)	4.43 ± 1.59	6.88 ± 3.88	2.45	-2.18	28	0.040
ODWB (kg)	3.85 ± 1.53	6.00 ± 3.49	2.15	-6.15	28	< 0.001
TCFW	54.00 ± 9.25	81.82 ± 13.88	27.82	-6.46	28	< 0.001
TCADW	33.20 ± 6.25	54.90 ± 14.06	21.70	-5.46	19.32	< 0.001
TCODW	25.56 ± 5.43	43.99 ± 11.69	18.43	-5.54	19.77	< 0.001

Note. DBH = diameter at breast height, FH = full height, MH = merchantable height, FW = fresh weight, ADW = air-dry weight, ODW = oven-dry weight, L = leaves, B = branches, TC = total components

In contrast, relatively small variability was found in the canopy components. Fresh weight of leaves (FWL) showed a much smaller mean difference of only 0.76 kg between two diameter classes, showing that diameter changes heavily influence main stem expansion rather than canopy development.

Regardless of diameter class, the correlation between DBH and FW for total aboveground biomass ($R^2 = 0.86$) and its individual components were consistently stronger than that for their corresponding ADW and ODW. Among all tree components, the pooled aboveground biomass exhibited the highest coefficient of determination, indicating a highly robust relationship with overall biomass accumulation. In contrast, the leaf component yielded the lowest R^2 value (0.39 - 0.47), reflecting a higher natural variability in canopy foliage compared to the more predictable accumulation of woody stem biomass (Figure 3).

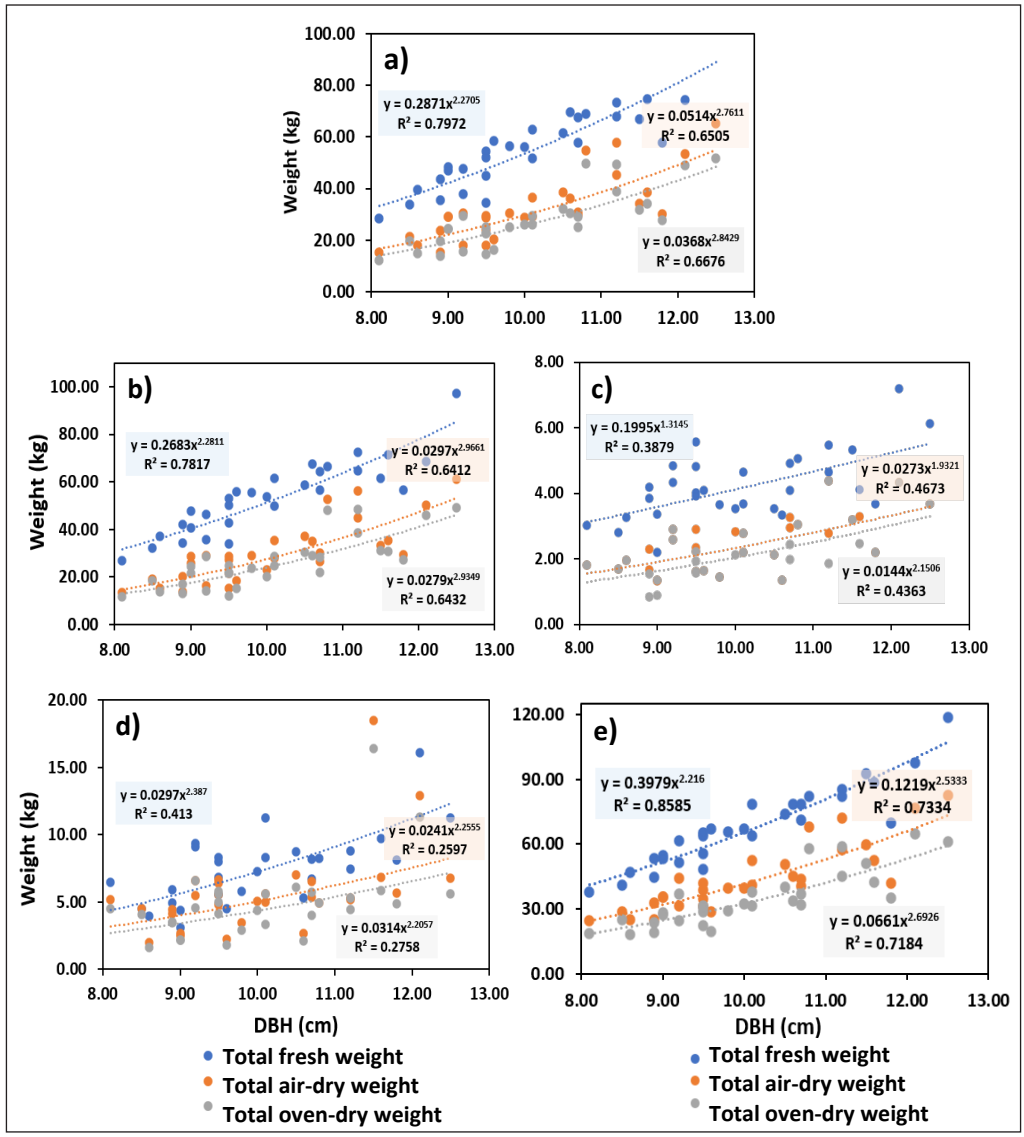


Figure 3. Relationship between diameter at breast height (DBH) and fresh, air-dry, and oven-dry weights for: a) full height logs, b) merchantable height logs, c) leaves, d) branches, and e) total aboveground biomass

Allometric Equation by Log Position

Figure 4 illustrates the relationship between the exponent (*b*) and the coefficient of determination (R^2) across different hydration states (FW, ADW and ODW) as well as log positions (top, middle and bottom) for FH and MH. The scatter plot reveals three distinct groups based on their *b* value and R^2 values. The top log in the FW state expresses a high *b* value and R^2 value, suggesting a well-defined relationship between DBH and biomass.

Nonetheless, the top log expresses the lowest *b* values (1.42-2.09) and weak correlations (R^2 = 0.18-0.33) at the ADW and ODW states.

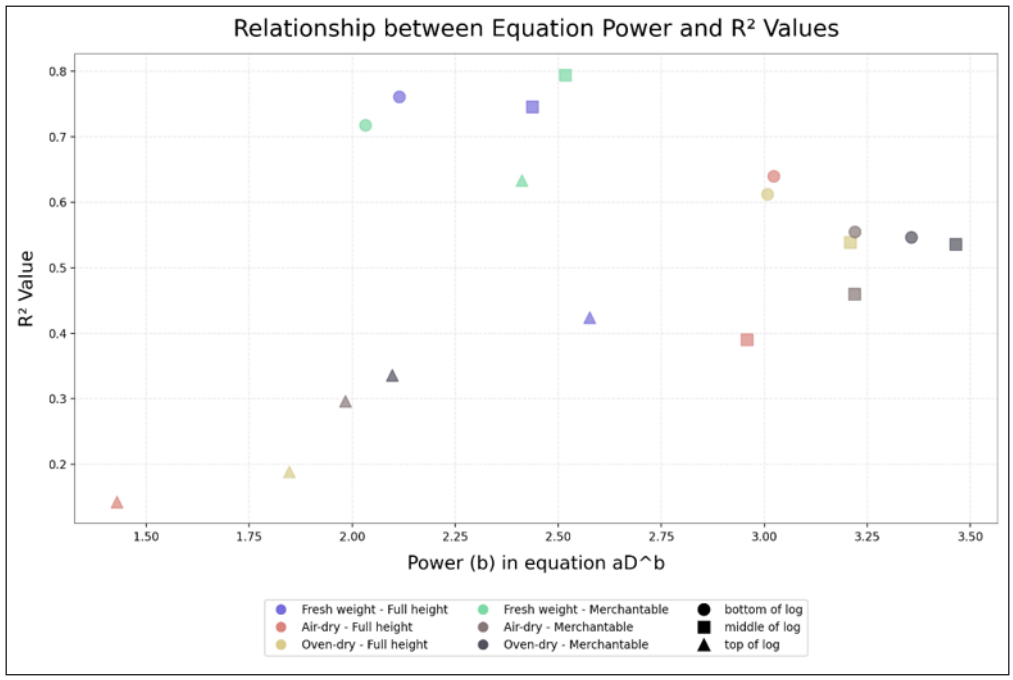


Figure 4. The relationship between the exponent (*b*) and the coefficient of determination (R^2) across different hydration states (fresh, air-dry, oven-dry) and log positions (top, middle, bottom)
Note. Each point is annotated with its corresponding equation value, and different colours and markers indicate the measurement type and position on the log

In contrast, the middle and bottom log sections exhibited a steadier distribution. Moderate *b* values (2.03-2.51) with moderate correlation (R^2= 0.63 - 0.79) for the FW state suggested a more consistent relationship between DBH and biomass. For ADW and ODW states, similar log positions showed the highest *b* values (2.95-3.35) with moderate correlations (R^2= 0.39 - 0.63), suggesting that biomass allocation in these sections is more predictable. Regardless of biomass allocation or log position, the allometric exponent (*b*>1) reliably exhibits a positive allometric relationship.

Volume for Log Interval and Form Factor Estimation

Newton's formula was adopted to establish an accurate baseline for the observed volume, involving the determination of independent tree variables (DBH and total height). The log volume and log length formed a clear sigmoidal relationship, reflecting the tree's tapering profile and specific wood allocation. The observed and predicted values fit the data well. Smalian's formula obtained the highest R^2(0.771) followed by Newton's (0.770), Huber's

(0.769) and Bruce's (0.759) (Figure 5). Meanwhile, Huber's formula was found to be the most accurate formula, confirmed by the lowest error estimates (Table 4). Specifically, Huber's Mean Bias Error (MBE) and Root Mean Square Error (RMSE) ranged from a minimal magnitude of 10^{-7} m^3 for the lower 0.3 m log intervals to a maximum of 10^{-4} m^3 for the highest log intervals (≥11.3 m).

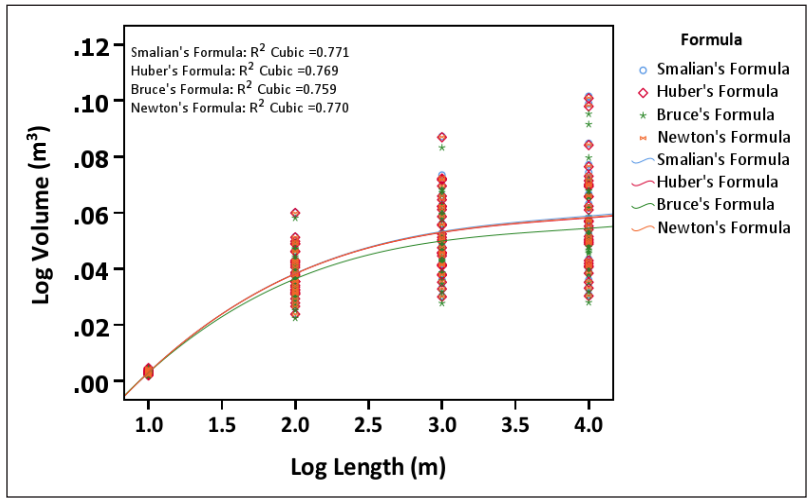


Figure 5. Predicted log volume against log length using Smalian's, Huber's, and Bruce's formulas

Table 4
Statistics of fit of the volume equations tested for 4-year-old Eucalyptus hybrid DH3229

Log Interval/ Formula	MBE (m^3)	RMSE (m^3)	SD (m^3)
0.3 m log interval			
Smalian	-6.10×10^{-7}	6.14×10^{-7}	6.97×10^{-8}
Huber	3.05×10^{-7}	3.07×10^{-7}	3.49×10^{-8}
Bruce	5.17×10^{-5}	5.20×10^{-5}	6.06×10^{-6}
≤ 5.3 m log interval			
Smalian	-7.53×10^{-5}	8.20×10^{-5}	3.32×10^{-5}
Huber	3.76×10^{-5}	4.10×10^{-5}	1.66×10^{-5}
Bruce	1.82×10^{-3}	1.86×10^{-3}	4.13×10^{-4}
9.3 m log interval			
Smalian	-2.29×10^{-4}	2.75×10^{-4}	1.54×10^{-4}
Huber	1.14×10^{-4}	1.37×10^{-4}	7.72×10^{-5}
Bruce	3.16×10^{-3}	3.22×10^{-3}	6.37×10^{-4}
≥ 11.3 m log interval			
Smalian	-4.43×10^{-4}	4.67×10^{-4}	1.52×10^{-4}
Huber	2.22×10^{-4}	2.34×10^{-4}	7.62×10^{-5}
Bruce	3.83×10^{-3}	3.91×10^{-3}	7.88×10^{-4}

The form factor of the individual tree for four-year-old *E.* hybrid DH3229 remained relatively stable across the sampled diameter range (8.10 cm to 12.50 cm). The analysis yielded a mean form factor of 0.579 ± 0.043, with values ranging from 0.480 to 0.704 (Figure 6).

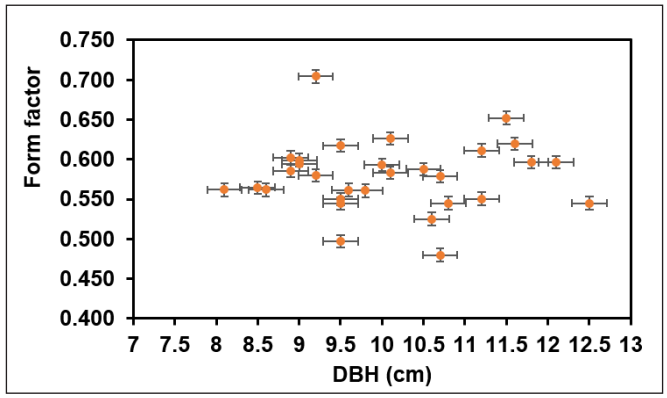


Figure 6. Tree individual form factors and diameter at breast height (DBH) relationships for 30 destructively sampled four-year-old *Eucalyptus* hybrid DH3229 trees

DISCUSSIONS

Biomass Estimation and Hydration Dynamics of *Eucalyptus* Hybrid DH3229

Topographical conditions and low-maintenance silviculture of the study site suggested the weak correlation observed between DBH and total tree height (R^2 = 0.097). Caldeira et al. (2023) studied the mean basal area of 3.5-year-old *Eucalyptus* trees were higher (35 $m^2 ha^{-1}$) at the bottom of the slope compared to the trees at the top of the slope (27 $m^2 ha^{-1}$). A higher significant correlation was found between the DBH variable and volume (r = 0.92, $p < 0.001$) than those between the H variable and volume (r = 0.82, $p < 0.001$) at four years of age of *Eucalyptus* hybrid was also reported by Wu et al. (2011). This high variability in height growth justifies relying on the DBH variable in biomass prediction.

While a sample size of 30 trees represents a structural limitation, normal distribution of DBH range and the highly robust coefficient of determination (R^2= 0.86) for total aboveground fresh biomass, confirming the sufficiency of sample size to capture stable allometric relationships. This approach is well-supported by a previous study by Abdul-Hamid et al. (2022), which successfully developed a single-variable DBH allometric equation for a natural mangrove forest by destructively harvesting 15 tree samples, yielding R^2= 0.9892 when validated to 1000 stand data trees.

Aboveground biomass of trees with DBH >10 cm exhibited approximately twice as high than those with DBH ≤10 cm. Although fast-growing species prioritise vertical development

over horizontal expansion, larger tree diameter still accumulates greater biomass due to allometric relationships in tree volume, which is influenced by both diameter and height (Abdul-Hamid et al., 2022). Wu et al. (2011) reported that 19 hybrids of *Eucalyptus* exhibited different growth traits and wood properties between clones and sites in China. The exponential scaling between diameter and biomass renders DBH a more influential factor in biomass accumulation than height (Mohamad Ismail et al., 2020).

Small variation between FW, ADW, and ODW at full height (FH; R^2 = 0.7972) and merchantable height (MH; R^2= 0.7817) observed were attributed to the canopy development and non-merchantable stem tip (Abiri et al., 2020; Wirabuana et al., 2021). The insignificant R^2 values observed between FH and MH suggest only a slight increment in biomass of the highly tapered upper log of FH. These findings emphasise that stem taper remains a relevant factor to consider in biomass estimation for both upper and lower logs, even in fast-growing species like *Eucalyptus*.

The FW models demonstrated greater performance ($R^2 \approx 0.80$) across all log positions compared to ADW and ODW weight models. In its fresh state, the measurement was done under highly controlled, uniform conditions (same age, same day, same site), which minimises the moisture variation and sample deterioration. Zhuang et al. (2024) studied the effects of water treatment on plant growth and reported R^2 values of 0.95 and 0.98 for two power functions used to establish allometric equations. On the contrary, the ADW exhibited a 32%-38% reduction compared to FW, while ODW declined progressively, exhibiting a 46%-52% reduction from FW and a 20%-24% reduction from ADW.

Allometric Equation Using aD^b for Top, Middle, and Bottom Parts of the Log

The drying process revealed distinct positional fluctuations in model accuracy. Despite moderate R^2 values (0.60 - 0.70) of middle and bottom logs in the dried states, the top log predictability declined significantly. This positional variance is further supported by the allometric exponents (b). The b value consistently demonstrated positive allometry ($b > 1$) across all measurements, but the lowest b value was yielded in the top log sections for both dried models. This reveals the stem taper effect, where progressive diameter reduction resulted in minimised biomass allocation in apical regions (Poorter et al., 2012), whilst highest b values confirmed primary biomass allocation to the lower stem segments.

Intrinsically, the dried models explained only ≤60% of biomass variation, implying that predictability is influenced by physiological, environmental and anatomical factors, including wood density, age-related and position-specific moisture dynamics during drying (Elustondo et al., 2023; Khan et al., 2025; Ribeiro et al., 2015). Top logs contain younger, less lignified tissue with predominant sapwood (characterised by higher initial moisture content), while middle and bottom logs feature greater proportions of dense heartwood with lower initial moisture content (Ren et al., 2023). This structural gradient

creates non-uniform drying responses, with top logs undergoing more substantial physical transformations during moisture loss, thereby reducing the stability of dry-weight predictions compared to the more robust FW estimates.

Eucalyptus Hybrid Volume and Form Factor Estimation

The sigmoidal relationship between log volume and log length highlights progressive volumetric changes, attributable to tree tapering. Despite the high sensitivity of Smalian's formula for shorter logs (0.3-5.3 m), it consistently underestimated volumes as the log position approached the canopy due to its inability to capture pronounced stem taper. In contrast, as stem taper became more pronounced, Huber's formula maintained consistent accuracy with minimal error, thereby minimising bias in whole-stem volume estimates. Particularly, the RMSE and SD by Huber's formula demonstrated higher precision than values reported by Hernández-Ramos et al. (2017) for *E. urophylla*. This indicates a substantial methodological improvement in volume prediction accuracy and consistency across log positions.

The current study sufficiently quantified the aboveground biomass and a reliable preliminary form factor for four-year-old *E.* hybrid. Based on the integration of 30 destructively sampled trees, real volume estimation employing Newton's formula resulted in a form factor of 0.579, surpassing the values reported in earlier studies. Corresponding studies for older (five- to nine-year-old) and larger DBH classes of *Eucalyptus* species by Japarudin et al. (2020) recorded form factors for *E. grandis*, *E. pellita*, *E. urophylla* and *E*. hybrid were in the range 0.365 to 0.379 in Asia while 0.466 was reported in Africa (Gezahagn et al., 2020). This disparity primarily reflects the differences in tree age and diameter ranges, emphasising the importance of age-specific quantification; younger, fast-growing trees possess different stem tapers and volume distributions before reaching optimal mature wood density (Curvo et al., 2024).

Study Limitation and Scope of Application

While this study provides valuable baseline allometric equations and empirical form factors for early-age *Eucalyptus* hybrid DH3229, there are methodological constraints inherent to the experiment design that must be addressed. First and foremost, the destructive sampling was based on a sample size of 30 trees harvested from a single plantation site. While a sample size of 30 trees spanning a stratified diameter range is practically sufficient for developing preliminary site-specific models, it intrinsically limits the broader statistical generalizability of the findings.

Furthermore, it is important to consider that the sampling was restricted to a single age class (four years old) under a low-intensity silvicultural management regime on sloped terrain. Consequently, the mathematical relationships and form factors determined here

reflect the specific growth dynamics, site quality, and microclimate of this study area. Therefore, these findings underscore the need for circumspection when applying these models to different geographic regions, varied silvicultural regimes, or older age classes. Although independent field validation across multiple sites was beyond the scope of the present study, empirical validation and testing across broader environmental gradients are strongly recommended before these models are employed as exploratory references for operational forest inventory or commercial yield projections in other settings.

CONCLUSION

Precise tree height measurements continue to present operational challenges. This study provides foundational information for early-age *Eucalyptus* hybrid DH3229 trees, showing that diameter at breast height (DBH) is the primary driver of biomass allocation, and that accurate biomass determination was obtained when measurements were taken in the fresh state. Even though the empirical form factor derived in this study is higher than those reported in previous studies, this finding emphasises the necessity of age- and site-specific quantification when developing mathematical expressions.

RECOMMENDATIONS

Future research should utilise a larger sample size and extend the study sites to encompass a wider array of geographical locations and early-age sequences (e.g., 2-5 years old) to refine these models. This will help clarify the developmental shifts in empirical form factors prior to rotation maturity. Furthermore, future studies should incorporate wood density variables and develop component-specific equations. This framework will reduce uncertainty in allometric relationships, offering reliable tools for short-rotation plantation inventory.

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Silico-Manganese Slag Application Ameliorates Acidity and Enhances Nutrient Availability in a Tropical Acidic Soil

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ABSTRACT

In tropical regions, excessive exchangeable acidity and aluminium (Al^{3+}) influence the availability of ammonium (NH_4^+), nitrate (NO_3^-), and phosphorus (P) because of low soil pH. This study focuses on the effects of silico-manganese slag (SiMn slag) and chemical fertilisers (CF) on mitigating acidity, exchangeable Al^{3+}, extractable manganese (Mn), and iron (Fe) for improving selected chemical properties [pH, NH_4^+, NO_3^-, P, and electrical conductivity (EC)] of a tropical acidic soil named Bekenu series (pH = 5.13). An incubation study was conducted by mixing one kilogramme of Bekenu series with different rates of SiMn slag and CF: T0 = soil only; T1 = SiMn slag only; T2 = soil + 100% CF; T3 = soil + 100% SiMn slag; T4 = soil + 75% SiMn slag + 25% CF; T5 = soil + 50% SiMn slag + 50% CF; and T6 = soil + 25% SiMn slag + 75% CF. After 30, 60, and 90 days of incubation, compared with T0, SiMn slag and CF significantly increased soil pH, exchangeable NH_4^+, available P, and EC, in addition to suppressing exchangeable acidity and Al^{3+}, as well as extractable Fe and Mn. This indicates that SiMn slag could be used as a liming amendment, whereas chemical fertilisers remain necessary for supplying nutrient sources. In future studies, the justification for fixing the suitable application rate of SiMn slag should focus on acid-neutralising capacity, calcium carbonate ($CaCO_3$) equivalent, target pH, exchangeable acidity neutralisation requirement, or silicon/calcium/magnesium (Si/Ca/Mg) supply. Future greenhouse and field trials are recommended to further validate the effects of SiMn slag on soil-plant interaction.

Keywords: Alkaline amendment, industrial by-product recycling, manganese alloy, neutralising ability, silicate amendment, soil acidity mitigation

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INTRODUCTION

In Malaysia, mineral acidic soils such as Ultisols and Oxisols occupy approximately 72% of the total land area because of excessive rainfalls and warm temperatures (Shamshuddin & Wan, 2011). Moreover, these Ultisols and Oxisols are made up of highly weathered iron rocks and clay minerals, which are characterised by low pH and excessive exchangeable acidity, limited nutrient retention and availability, low organic matter content, and high levels of iron (Fe) and aluminium (Al^{3+}), rendering them relatively infertile (Omar et al., 2021; Latifah et al., 2017). This phenomenon leads to the depletion of base cations such as potassium (K^{+}), calcium (Ca^{2+}), magnesium (Mg^{2+}), and sodium (Na^{+}) via leaching and surface runoff due to the substantial rainfalls (Ch'ng et al., 2014; Ng et al., 2021). The process is followed by the presence of soluble acidic cations, such as Al^{3+}, Fe^{3+}, and $H^{+,}$ because of continued decrease in soil pH, which could hamper soil and crop productivity (Fageria & Nascente, 2014; Ng et al., 2024a).

Therefore, addressing excessive soil acidity and exchangeable Al^{3+} is critical to improve soil and crop productivity in the tropical regions. Perumal (2016) and Hassan et al. (2023) suggested that enhancing soil fertility and implementing efficient nutrient management practices using agricultural wastes can improve crop yields, ensure food security, and elevate agricultural productivity in the highly weathered Ultisols and Oxisols. Moreover, Ch'ng et al. (2019) and Hasbullah et al. (2020) reported that the sole application of chemical fertilisers such as rock phosphate not only causes P fixation by exchangeable Al^{3+} and extractable Fe, but it also significantly increases P leaching. Thus, this approach is often economically and environmentally unsustainable, resulting in significant nutrient losses into the water bodies and polluting the environment via eutrophication. To fix the problem, amending the soil acidity with a suitable soil conditioner is essential to improve nutrient availability in addition to reducing the fertiliser application rate for achieving agronomic efficiency, economic viability, and environmental conservation.

A promising and novel strategy is crucial for enhancing the productivity of Malaysian soils, aimed at improving crop yields and economic income for both small and large-scale farmers using organic and inorganic soil conditioning amendments (Chong et al., 2022; Johan et al., 2021; Jusop & Ishak, 2010; Ali et al., 2023). One such amendment with the potential to augment soil physical, chemical, and biological properties is the incorporation of silico-manganese slag (SiMn slag) (Buruiana et al., 2021). It is reported that SiMn slag is a by-product generated during the production of silico-manganese alloys, which are primarily used in steel manufacturing (Wu et al., 2007). Notably, as a well-known industrial area, Bintulu in Sarawak, Malaysia, produced a massive amount of SiMn slag at approximately 444,000 tons annually in 2022 from smelting activities (Ting et al., 2023). Despite its industrial significance as construction and building materials, the full potential of SiMn slag, its properties, and its application in the agricultural sector to amend the chemical

properties of acidic soils in tropical regions remain unexplored. In this context, conducting localised research such as a soil incubation study can unveil valuable insights into the physico-chemical characteristics of SiMn slag to manage acidic soils. Wu et al. (2007) also reported that approximately 15% to 20% of total steel production resulted in the formation of SiMn slag. It is opined that SiMn slag is distinguished by its advantageous qualities, such as neutralising capability due to its alkalinity, ease of application, considerable amounts of alkaline materials such as silicon oxide (26.19% to 47.54%) (Mussawar et al., 2022; Wang et al., 2017), calcium oxide (9.24% to 29.10%) (Navarro et al., 2017; Navarro et al., 2020; Wang et al., 2017), and magnesium oxide (3.70% to 6.91%) (Cheng et al., 2023; Criado et al., 2018) contents.

Due to the considerable amounts of SiO_2, CaO, and MgO as well as the alkaline pH of SiMn slag, Tozsin and Oztas (2023) reported that slag is an industrial waste from steel production that could be used as a soil amendment and mineral fertiliser in agriculture. For example, excessive acidity amelioration and improved availability of plant nutrients such as P, sulphur (S), Mn, Fe, and molybdenum (Mo) in acidic soils may be associated with the inclusion of slag (Díaz-Piloneta et al., 2022; Prakash, 2023; Tozsin & Oztas, 2023). Islam et al. (2022) also revealed that the gradual increase of pH in acidic soils is consistent with the increasing amounts of steel slag, resulting in increased N-circulation and decreased P-circulation activities at four to six weeks after the cultivation of *Brassica rapa* var. *periviridis* and *Spinacia oleracea* L. Beyond its liming effects to improve soil pH, silicon (Si) in SiMn slag could detoxify soil from excessive Al^{3+} and extractable Fe in acidic soils, which can be detrimental to plant roots and inhibit P uptake by plants (Azman et al., 2023; Jo et al., 2022). The elevated soil pH could also increase P availability by reducing the concentrations of Al^{3+} and extractable Fe, which are transformed into insoluble hydroxides (Johan et al., 2022). Moreover, many studies such as Schaller et al. (2019), Ng et al. (2024b), and Chong et al. (2022) reported that the inclusion of silicate materials could have contributed to the increase in soil P availability for crop uptake. For example, Schaller et al. (2019) reported that the inclusion of silicate amendments could increase soil P availability when the release of water-soluble Si replaced and mobilised P at the adsorption sites of Fe minerals. The increased P availability in the soils with Si additions may be associated with the stronger binding affinity of Si to Fe minerals compared with P (Chong et al., 2022; Ng et al., 2024b; Schaller et al., 2022).

The potential effects of SiMn slag on improving selected chemical properties of tropical acidic soil over time remain unexplored. It was hypothesised that the combined use of SiMn slag and CF could increase pH, exchangeable NH_4^+, available NO_3^- and P, and EC through the suppression of exchangeable acidity, Al^{3+}, extractable Mn, and Fe, after 90 days of incubation. It was plausible that the optimum application rate of SiMn slag could neutralise exchangeable acidity and increase soil pH, which may be associated with the

presence of CaO, MgO, and SiO_3^{2-} besides reducing Al^{3+} and Fe. Therefore, the objective of this incubation assessment was to elucidate the effects of SiMn slag and chemical fertilisers on pH, exchangeable NH_4^+, available NO_3^- and P, EC, acidity, Al^{3+}, and extractable Mn and Fe, at 30, 60, and 90 days of incubation. The findings in this incubation study could enhance the understanding of the combined use of SiMn slag and chemical fertilisers in neutralising excessive acidity in addition to improving the nutrient availability (particularly for N and P) of highly weathered acidic soils in the tropical regions.

MATERIALS AND METHODS

Sampling and Preparation of Soil

The soil used in this incubation study was the Bekenu series (*Typic Paleudults*). It is a typical acidic soil used for oil palm, rubber, and black pepper plantations (Paramananthan, 2020). The soil sample was collected using a shovel from an uncultivated area in Universiti Putra Malaysia Sarawak (UPMS) (latitude 03°20 N, longitude 113°07 E) (Figure 1). According to Johan et al. (2021), the microclimate of the soil sampling area has an average annual rainfall, air temperature, and relative humidity of 2993 mm, 27 °C, and 80%, respectively. The soil sample was collected in a pedon (1 m length × 1m width) at a 0.2 m sampling depth, followed by air-drying, manual crushing, and passing through a 2.0 mm sieve prior to the determination of selected physico-chemical properties.



Figure 1. The location of the soil sampling site for the laboratory incubation study in Universiti Putra Malaysia Sarawak, Bintulu, Malaysia

Silico-Manganese Slag Preparation

The silico-manganese slag (SiMn slag) was sourced from Pertama Ferroalloys Sdn. Bhd., Bintulu, Sarawak, Malaysia (latitude 03°54 N, longitude 113°31 E), as shown in Figure 2. The SiMn slag was randomly collected into gunny sacks using a shovel and then transported to the Soil Science Laboratory under the Department of Crop Science in UPMS for air-drying, manual crushing, and passing through a 2.0 mm sieve before the physicochemical properties characterisation.



Figure 2. Field collection of SiMn slag at Pertama Ferroalloys Sdn. Bhd., Bintulu, Sarawak, Malaysia

Determination of Soil and Silico-Manganese Slag Physico-Chemical Properties

The texture of soil and particle size distribution of SiMn slag were determined using the hydrometer method (Bouyoucos, 1962). Soil bulk density was determined using the coring method (Tan, 2005). The pH in 1 mol dm^{-3} potassium chloride (pH in KCl), pH in water, and electrical conductivity (EC) were determined via potentiometric method using a digital pH meter (SevenEasy pH, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) and a digital EC meter (SevenEasy Conductivity, Mettler-Toledo GmbH, Schwerzenbach, Switzerland), respectively (Peech, 1965). Soil cation exchange capacity (CEC) was determined using the leaching method, followed by steam distillation (Bremner & Keeney, 1965; Cottenie, 1980). Soil exchangeable acidity and Al^{3+} were determined using the titration method (Rowell, 1994). The organic matter and carbon were determined using the loss on ignition method (Tan, 2005). The method of Keeney and Nelson (1982) was used to determine soil exchangeable NH_4^+ and available NO_3^- using steam distillation. Soil available P and cations such as exchangeable potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), and extractable Mn and Fe were extracted using Mehlich's double acid method (Mehlich, 1953). Available P was determined using Ultraviolet-Visible Spectrophotometry (Lambda 25, Perkin Elmer) after the blue colour development method (Murphy & Riley, 1962). Whereas exchangeable K^+, Ca^{2+}, Mg^{2+}, and extractable Mn and Fe were determined using

Atomic Absorption Spectrophotometry (AAnalyst 800, Perkin Elmer, CT). The silicon and manganese oxides of SiMn slag were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The prepared sample of SiMn slag was sent to an external laboratory (Chemsain Konsultan Sdn. Bhd., Kuching, Sarawak, Malaysia) for the determination of heavy metals such as lead (Pb), nickel (Ni), cadmium (Cd), chromium (Cr), mercury (Hg), and arsenic (As). The prepared sample was extracted using nitric acid and hydrochloric acid, followed by gentle heating (not higher than 85 °C) in accordance with the procedures outlined in United States. Environmental Protection Agency (EPA) 200.2 (EPA, 1994b) and determined using ICP-OES based on EPA Method 6010D (SW-846) described by EPA (2018), except for Hg using Cold Vapour AAS (EPA, 1994a). The elemental composition of SiMn slag was determined using Energy Dispersive X-ray (EDX) (JEOL JSM-IT500 HR).

Soil Incubation Experiment

A soil incubation experiment was carried out in the Soil Science Laboratory at the Department of Crop Science, Universiti Putra Malaysia Sarawak, Bintulu, Malaysia. One kilogramme (1000 g) of air-dried soil was weighed in a cylindrical plastic container (15 cm diameter × 12 cm height). After the soil physico-chemical characterisation, different rates of SiMn slag and chemical fertilisers were determined in accordance with Malaysian Agricultural Research and Development Institute (MARDI) recommendations for maize (*Zea mays* L.; Thai super sweet hybrid F1 variety) as our test crop in the future greenhouse and field experiments. The recommended rates of nitrogen (N), phosphorus (P), and potassium (K) sources supplied to the maize on a per-hectare basis are 60 kg ha^{-1} N; 60 kg ha^{-1} P_2O_5; 40 kg ha^{-1} K_2O (130 kg ha^{-1} urea; 130 kg ha^{-1} Triple Super Phosphate; 67 kg ha^{-1} Muriate of Potash), respectively (MARDI, 1993). In this soil incubation study, based on the planting density of approximately 26,667 maize plants per hectare (75 cm between the two rows × 50 cm between two plants within a row), the application rate of the mixture of straight fertilisers (urea, TSP, and MOP) was then scaled down to a per-plant basis at 12.26 g.

Afterwards, varying rates by 25% (100%, 75%, 50%, 25%, and 0%) on the combined use of chemical fertilisers and SiMn slag were used to determine the effects of treatments on the selected soil chemical properties and to reduce the reliance on chemical fertilisers in maize cultivation. Thus, the rates of the SiMn slag and chemical fertilisers were varied at 12.26 g (100%), 9.20 g (75%), 6.13 g (50%), and 3.07 g (25%). In this soil incubation study, the varying application rates of SiMn slag were fixed to replace the reduced amounts of chemical fertilisers by every 25% via mass substitution approach to determine the most suitable combination of SiMn slag and chemical fertilisers to mitigate acidity and improve nutrient availability in the Bekenu series. It was noted that the changes in percentages via the

mass substitution approach are experimental mass-mixture proportions and do not represent nutrient-equivalent replacement rates. Moreover, there were two additional treatments of soil only (T0) and SiMn slag only (T1) to elucidate the longevity of the treatment effects over time. Therefore, there were a total of seven treatments for each batch of the incubation. The treatments evaluated in this soil incubation experiment are summarised as follows:

T0 : 1 kg soil only
T1 : 1 kg silico-manganese slag only (SiMn slag)
T2 : 1 kg soil + 12.26 g chemical fertilisers (100%)
T3 : 1 kg soil + 12.26 g SiMn slag (100%)
T4 : 1 kg soil + 9.20 g SiMn slag (75%) + 3.07 g chemical fertilisers (25%)
T5 : 1 kg soil + 6.13 g SiMn slag (50%) + 6.13 g chemical fertilisers (50%)
T6 : 1 kg soil + 3.07 g SiMn slag (25%) + 9.20 g chemical fertilisers (75%)

The soil, SiMn slag, and chemical fertilisers were thoroughly mixed inside the container. Thereafter, the mixture of each treatment was moistened with distilled water at 40% to 60% water holding capacity (field capacity) of the soil and SiMn slag. Water holding capacity was determined by pouring 100 mL of distilled water onto a 100 g air-dried sample in a filter funnel, where its base was placed with a piece of filter paper to prevent the leaching of soil particles. A 100 mL measuring cylinder was placed under the filter funnel to determine the volume of water leached out from the soil, and the moistened soil was left for two hours. After two hours, the volume of water retained in the soil was known as water holding capacity, whereas 40% to 60% of the water holding capacity is known as field capacity. To fix the volume of distilled water to be added in each treatment, the distilled water was added meticulously until the entire soil mixture was moistened, after which the moistened sample was covered with a lid perforated with two holes (approximately 7 mm in diameter) to enable good aeration and prevent moisture loss from the mixture, as shown in Figure 3.

Throughout the experiment, the soil moisture was monitored every week by observing the presence of water droplets on the surface of the container within the enclosed soil system. A 5 mL of distilled water was meticulously sprayed onto each soil sample when the water droplets on the surface of the containers disappeared to maintain the soil moisture. The soil samples were incubated for 30, 60, and 90 days of incubation (DAI), respectively. At the end of the experiment, the incubated soils for each batch were separately and destructively sampled at each incubation time. Thereafter, the collected soil samples were air-dried, manually crushed, and passed through a 2 mm sieve for chemical analyses, which included pH in KCl, pH in water, exchangeable acidity, Al^{3+}, NH_4^+, available NO_3^- and P, extractable Mn, Fe, and EC.



Figure 3. Layout of laboratory incubation experiment

Experimental Design and Statistical Analysis

The soil incubation study was a 7 × 3 factorial experiment with three replicates per treatment, arranged in a Completely Randomised Design (CRD). There were seven treatments for each incubation period and three sampling times (30, 60, and 90 days of incubation) in this experiment. The collected data were analysed using a normality test to determine if the data were well-fitted to a normal distribution. Afterwards, the data were analysed using two-way Analysis of Variance (ANOVA) to determine the effects of treatment and sampling time, followed by mean comparisons using Tukey's HSD test at $p \leq 0.05$. The software used was the Statistical Analysis System (SAS) version 9.4, Cary, NC, USA.

RESULTS AND DISCUSSION

Initial Physico-Chemical Properties of Soil and Silico-Manganese Slag

Table 1 presents the selected physico-chemical properties of the Bekenu series. It was a sandy clay loam with the proportions of sand, silt, and clay particles at 48.3%, 24.0%, and 27.7%, respectively. The soil had a bulk density of 1.20 g cm^{-3}, which is an ideal bulk density for plant growth in accordance with Nyéki et al. (2017). According to Ng et al. (2021), the parent materials of the Bekenu series are red-yellow sedimentary rocks, resulting in a soil pH in water of 5.13 (acidity level: strongly acidic), where the excessive rainfall and weathering may result in low soil CEC (7.70 $cmol_{(+)}kg^{-1}$) and EC (90 µS cm^{-1}). When the soil pH is lower than 5.5, it has an excessive exchangeable acidity (1.21 $cmol_{(+)}kg^{-1}$) and exchangeable Al^{3+} (1.03 $cmol_{(+)}kg^{-1}$), which exceeded the Al^{3+} toxicity

threshold of 0.80 $cmol_{(+)}kg^{-1}$, and this can significantly hinder crop productivity due to impaired root growth (Ch'ng *et al.,* 2014). Moreover, the Al^{3+} toxicity not only causes P fixation (available P = 1.24 mg kg^{-1}), but the phenomenon also causes significant leaching of exchangeable cations such as K^+ (76.0 mg kg^{-1}), Ca^{2+} (17.1 mg kg^{-1}), and Mg^{2+} (20.4 mg kg^{-1}).

The initial physico-chemical properties of SiMn slag are summarised in Table 2. The SiMn slag had a particle size distribution of 57.32% (0.05 mm to 2.0 mm), 4.58% (0.002 mm to 0.05 mm), and 38.10% (<0.002 mm), respectively. The SiMn slag was an alkaline material (pH = 8.00) which had higher concentrations of exchangeable base cations such as K^+ (240.8 mg kg^{-1}), Ca^{2+} (1391.6 mg kg^{-1}), and Mg^{2+} (496.5 mg kg^{-1}). Conversely, the exchangeable Al^{3+} of the amendment was low at 0.16 $cmol_{(+)}$ kg^{-1}. Furthermore, it was found that SiMn slag is a mineral amendment because of its low total organic matter (4.00%). The amendment was low in exchangeable ammonium (0.7 mg kg^{-1}), available nitrate (1.0 mg kg^{-1}), and available P (0.9 mg kg^{-1}), suggesting that additional N and P fertilisers are crucial to compensate for the insufficient supplies of N and P for optimum maize growth.

Table 1
Initial physico-chemical properties of Bekenu series

Physico-chemical Properties	Unit	Value Obtained
Soil texture (USDA)	%	Sand: 48.3 Silt: 24.0 Clay: 27.7 Sandy clay loam
Bulk density	g cm^{-3}	1.20
Soil pH in water	-	5.13
Electrical conductivity	µS cm^{-1}	90
Cation exchange capacity	$cmol_{(+)}kg^{-1}$	7.70
Exchangeable acidity		1.21
Exchangeable Al^{3+}		1.03
Exchangeable NH_4^+	mg kg^{-1}	28.02
Available NO_3^-		15.88
Available P		1.24
Exchangeable K^+		76.0
Exchangeable Ca^{2+}		17.1
Exchangeable Mg^{2+}		20.4

The silicon oxide (SiO_2) and manganese oxide (MnO) of SiMn slag were 27.3% and 13.74%, respectively. The presence of SiO_2 in the SiMn slag used in this study is in agreement with the findings of Mussawar et al. (2022) and Wang et al. (2017) who reported that the SiO_2 of SiMn ranges from 26.19% to 47.54%, whereas the obtained value for MnO in SiMg slag was slightly higher compared with the reported range of 9.90% to 12.23% by Navarro et al. (2017) and Kumar et al. (2013). Therefore, the use of SiMn slag can increase soil pH because of its alkalinity, but the silicon in SiMn slag could also mobilise P, as reported by Schaller et al. (2019). Based on Table 2, it was observed that the concentrations of lead (2.3 mg kg^{-1}), nickel (8.9 mg kg^{-1}), cadmium (not detected), chromium (4.2 mg kg^{-1}), mercury (not detected), and arsenic (4.6 mg kg^{-1}) were lower

than the contamination thresholds of 36 mg kg^{-1}, 28.9 mg kg^{-1}, 0.09 mg kg^{-1}, 14.4 mg kg^{-1}, 0.42 mg kg^{-1}, and 43 mg kg^{-1}, respectively (Department of Environment Malaysia, 2009). This finding suggests that the measured total concentrations of heavy metals in SiMn slag were below the cited screening values, although studies such as leaching, plant uptake, residual effects due to continuous applications, and long-term accumulation were not evaluated in this study. Therefore, it is recommended that other experiments such as leaching and plant bioassay studies should be commenced to determine the residual effects over continuous applications and heavy metal uptake by the plants. In terms of elemental composition, SiMn slag is made up of the elements in the descending order: oxygen (56.39%), silicon (17.37%), carbon (12.70%), calcium (10.21%), magnesium (2.48%), and potassium (0.84%) (Table 3). However, it is was noted that Mn remains undetectable in the EDX analysis.

Table 3
Elemental composition of silico-manganese slag analysed using Energy Dispersive X-ray

Elemental composition	Mass (%)
C	12.70±0.09
O	56.39±0.28
Mg	2.48±0.07
Si	17.37±0.18
K	0.84±0.09
Ca	10.21±0.23
Total	100.00

Co-Application of Silico-Manganese Slag and Chemical Fertilisers on Soil pH, Exchangeable Acidity and Aluminium, Extractable Iron and Manganese

Soil pH in water for the soil with co-application of SiMn slag (25%) and chemical

Table 2
Selected physico-chemical properties of silico-manganese slag

Physico-chemical Properties	Unit	Value Obtained
Particle size distribution	%	0.05-2.0 mm: 57.32 0.002-0.05 mm: 4.58 <0.002 mm: 38.10
Total organic matter		4.00
Total organic carbon		2.32
Silicon oxide		27.3
Manganese oxide		13.74
Soil pH in water	-	8.00
Electrical conductivity	µS cm^{-1}	360
Cation exchange capacity	$cmol_{(+)}$ kg^{-1}	12.00
Exchangeable Al^{3+}		0.16
Exchangeable NH_4^+	mg kg^{-1}	0.7
Available NO_3^-		1.0
Available P		0.9
Exchangeable K^+		240.8
Exchangeable Ca^{2+}		1391.6
Exchangeable Mg^{2+}		495.6
Lead (as Pb)		2.3
Nickel (as Ni)		8.9
Cadmium (as Cd)		Not detected (<1.5)
Chromium (as Cr)		4.2
Mercury (as Hg)		Not detected (<0.1)
Arsenic (as As)		4.6

fertilisers (75%) (T6) significantly and consistently increased to 6.32 at 30 DAI and 6.30 at 60 DAI compared with soil without any amendments (T0) at 5.73 (30 DAI) and 5.56 (60 DAI) (Table 4 and Figure 4a). For pH in KCl, the soil treated with SiMn slag (25%) and chemical fertilisers (75%) (T6) showed substantially higher pH in KCl of 4.92 at 30 DAI, 4.89 at 60 DAI, and 4.85 at 90 DAI, when compared with the untreated soils (T0) at 30 DAI (4.29), 60 DAI (4.01), and 90 DAI (4.03) (Table 4 and Figure 4b). Notably, compared with T0, it was observed that the co-application of SiMn slag and chemical fertilisers (T4, T5, and T6) significantly increased soil pH in water and KCl at 60 DAI. Moreover, the sole application of chemical fertilisers (T2) did not significantly increase the soil pH in water and KCl over 90 days of incubation, suggesting that the alkalinity of SiMn slag could significantly increase soil pH because the pH in water and KCl of SiMn slag only (T1) were consistently alkaline.

However, at 90 DAI, regardless of the varying rates of SiMn slag and chemical fertilisers, the soil pH in water and KCl in most of the treatments did not show significant differences compared with T0, suggesting that the effects of SiMn slag on soil pH in water and KCl might only last for 60 days and an additional application of SiMn slag is needed to improve and maintain the soil pH over time. This finding was further supported by the observations, which demonstrate that soil pH in water and KCl in T2, T4, and T5 significantly decreased at 90 DAI compared with those at 30 DAI (Table 4). On the contrary, soil pH in water and KCl in T3 and T6 were consistently stable over a 90 days of incubation period.

Because soil pH is negatively correlated with soil exchangeable H^+ concentration, the increase in pH in the amended soils may be associated with a considerable decrease in soil exchangeable H^+. This finding is consistent with the findings of Cai et al. (2015) who reported that adding amendments with a higher base saturation compared with the acidic soils could improve pH to the optimum range via the immobilisation of H^+ through ionic exchange interactions such as K^+, Ca^{2+}, and Mg^{2+} between the amendments and soil colloids. Moreover, the significant increase in soil pH may be associated with the neutralisation of H^+ by calcium oxide (CaO) in SiMn slag, where one mole of CaO reacts with two moles of H^+ to produce Ca^{2+} and water molecules (Navarro et al., 2017; Navarro et al., 2020; Wang et al., 2017). Furthermore, the dissolution of SiMn slag may have released silicate ions, which react with water molecules to form monosilicic acid (H_4SiO_4) via hydrolysis (Sahebi et al., 2015). According to Chong et al. (2022), this hydrolysis reaction could have released OH^-, which can increase soil pH and buffering capacity. However, the ineffectiveness of SiMn slag on increasing soil pH at 90 DAI in T3, T4, T5, and T6 could be related to the slow dissolution of SiMn slag into water-soluble silicon. Therefore, it is recommended that the combined use of SiMn slag and a water-soluble source of silicon such as sodium silicate could be used to improve the effects and extend the longevity of the treatments over time in further studies.

Effects of SiMn slag on neutralising exchangeable acidity in T3, T4, T5, and T6 were not pronounced at 30 DAI compared with T0 (Figure 4c). Moreover, at 30 DAI, the exchangeable acidity in T2 (soil + 100% CF) (0.67 $cmol_{(+)}\ kg^{-1}$) was significantly lower compared with T0 (1.22 $cmol_{(+)}\ kg^{-1}$), which may be associated with urea hydrolysis (Hamidi et al., 2021). However, compared with T0, the sole application of CF (T2) did not significantly reduce exchangeable acidity at 60 and 90 DAI. The soils treated with the SiMn slag (T3, T4, and T5) showed significantly lower exchangeable acidity in the range of 0.46 to 0.64 $cmol_{(+)}kg^{-1}$ at 90 DAI (Figure 4c). In comparison with the untreated soils (T0) at 60 DAI (1.17 $cmol_{(+)}kg^{-1}$), the soils treated with 6.13 g SiMn slag (50%) + 6.13 g chemical fertilisers (50%) (T5) showed significantly lower exchangeable acidity (0.72 $cmol_{(+)}kg^{-1}$). In terms of the effects of sampling time, the results indicate that the exchangeable acidity in T3, T4, and T5 was significantly lower at 90 DAI compared with that at 30 and 60 DAI (Table 5). Conversely, exchangeable acidity in T2 and T6 significantly increased at 90 DAI compared with that at 30 and 60 DAI. These findings indicate that the increase in soil pH was not the dominant factor that influenced the exchangeable acidity in this soil incubation study.

Compared with T0, some of the treatments containing the co-application of SiMn slag and CF had significantly lower exchangeable Al^{3+} ranged from 0.23 to 0.47 $cmol_{(+)}\ kg^{-1}$ at 30 DAI (T3, T4, and T5) and 0.14 $mol_{(+)}\ kg^{-1}$ at 60 DAI (T6), respectively (Figure 4d). These values were below the threshold of Al toxicity at 0.8 $cmol_{(+)}\ kg^{-1}$. These findings could be related to the use of SiMn slag in transforming exchangeable Al^{3+} into insoluble Al hydroxides via precipitation, which may be associated with the significant increase in soil pH (Moir & Moot, 2014; Yerima et al., 2020). Pavlů et al. (2021) also opined that the hydrolysis process of exchangeable Al^{3+} to produce H^+ could be impaired with increasing soil pH. For instance, three moles of H^+ are produced when one mole of exchangeable Al^{3+} is fully hydrolysed, further lowering the soil pH. Moreover, Jo et al. (2022) also pointed out that the addition of Si could have stimulated the reaction between $Al(OH)_3$ and Si into stable aluminium silicate ($Al_2O_3 \cdot 6SiO_2$), thus preventing the reverse reaction of $Al(OH)_3$ into toxic Al^{3+}. Therefore, we concluded that the alkalinity of SiMn slag could be plausible to ameliorate soil exchangeable acidity and precipitate active Al^{3+} into insoluble Al hydroxide, which may have prevented this reaction from producing more H^+. In terms of the effects of sampling time, except for T3, similar findings were observed where the exchangeable Al^{3+} was significantly reduced in T4 and T5, whereas exchangeable Al^{3+} in T2 and T6 was significantly higher at 90 DAI (Table 5). Further studies on the transformation of clay mineralogy or ionic speciation in the soil following the application of SiMn slag are recommended to validate our observations in this soil incubation study.

Regardless of sampling time, the application of SiMn slag alone (T3) and the co-application of SiMn slag and chemical fertilisers (T4, T5, and T6) significantly reduced

extractable Mn compared with soil without any amendments (T0) (Table 4 and Figure 4e). Notably, the SiMn slag only (T1) treatment demonstrated the lowest extractable Mn at 30 DAI (0.61 mg kg^{-1}), 60 DAI (0.76 mg kg^{-1}), and 90 DAI (0.81 mg kg^{-1}), suggesting that the inclusion of SiMn slag did not increase extractable Mn over 90 days of incubation conditions. The reduced extractable Mn in the soils with the application of SiMn slag alone (T3) and the co-application of SiMn slag and chemical fertilisers (T4, T5, and T6) could also be associated with the significant change in soil pH and adsorption of extractable Mn on the applied SiMn slag. It was reported that the soil extractable Mn could be significantly reduced through a significant increase in soil pH, which transforms exchangeable Mn^{2+} into Mn (IV) oxide fraction via oxidation process (De Schamphelaire et al., 2007; Fischel et al., 2015; Mayanna et al., 2015).

The application of SiMn slag alone (T3) and the co-application of SiMn slag and chemical fertilisers (T4, T5, and T6) significantly reduced extractable Fe (Table 4) to the range of 0.70 to 1.07 mg kg^{-1} at 60 DAI and 0.84 to 1.15 mg kg^{-1} at 90 DAI compared with T0 at 60 DAI (1.92 mg kg^{-1}) and 90 DAI (1.38 mg kg^{-1}), respectively (Figure 4f). This is because the elevated soil pH could have transformed extractable Fe into insoluble Fe hydroxides when it reacts with OH^- (Ng et al., 2022). Also, the dissolution of SiMn slag over time might have released SiO_3^{2-} to react with extractable Fe to form an insoluble monomolecular film ($FeOSi(OH)_3^+$) through anodic reaction (Kudryavtsev et al., 2016). Khan et al. (2024) reported that drying of moistened soils might have potentially altered

Table 4
Two-way analysis of variance (ANOVA) results to determine the effects of treatment, sampling time, and interaction of treatment and sampling time on soil pH, exchangeable acidity and aluminium, and extractable manganese and iron

Source of Variations	**Treatment**	**Time**	**Treatment* Time**	**Error**
Degree of freedom	6	2	12	42
Variables	F value (p-value)			
pH in water	139.44* (<0.0001)	15.34* (<0.0001)	1.31^{ns} (0.2466)	-
pH in KCl	557.11* (<0.0001)	10.23* (0.0002)	1.11^{ns} (0.3777)	-
Exch. acidity ($cmol_{(+)}$ kg^{-1})	24.01* (<0.0001)	34.97* (<0.0001)	36.57* (<0.0001)	-
Exch. Al^{3+} ($cmol_{(+)}$ kg^{-1})	80.79* (<0.0001)	35.62* (<0.0001)	108.60* (<0.0001)	-
Extractable Mn (mg kg^{-1})	7937.84* (<0.0001)	4.61* (0.0154)	4.03* (0.0004)	-
Extractable Fe (mg kg^{-1})	3763.01* (<0.0001)	3203.04* (<0.0001)	398.74 (<0.0001)	-

Note. *indicates significant effects, whereas ^{ns} denotes no significant effect at $p \leqslant 0.05$

the redox-sensitive metals such as Mn and Fe. For example, the addition of water to dried soils might have stimulated the solubilisation of micronutrients such as Mn and Fe, suggesting that the commencement of redox reaction studies for such elements is worth considering. It is noteworthy to recommend that sorption and plant bioassay studies should be commenced to elucidate the change in extractable Mn and Fe in the soil following the application of SiMn slag.

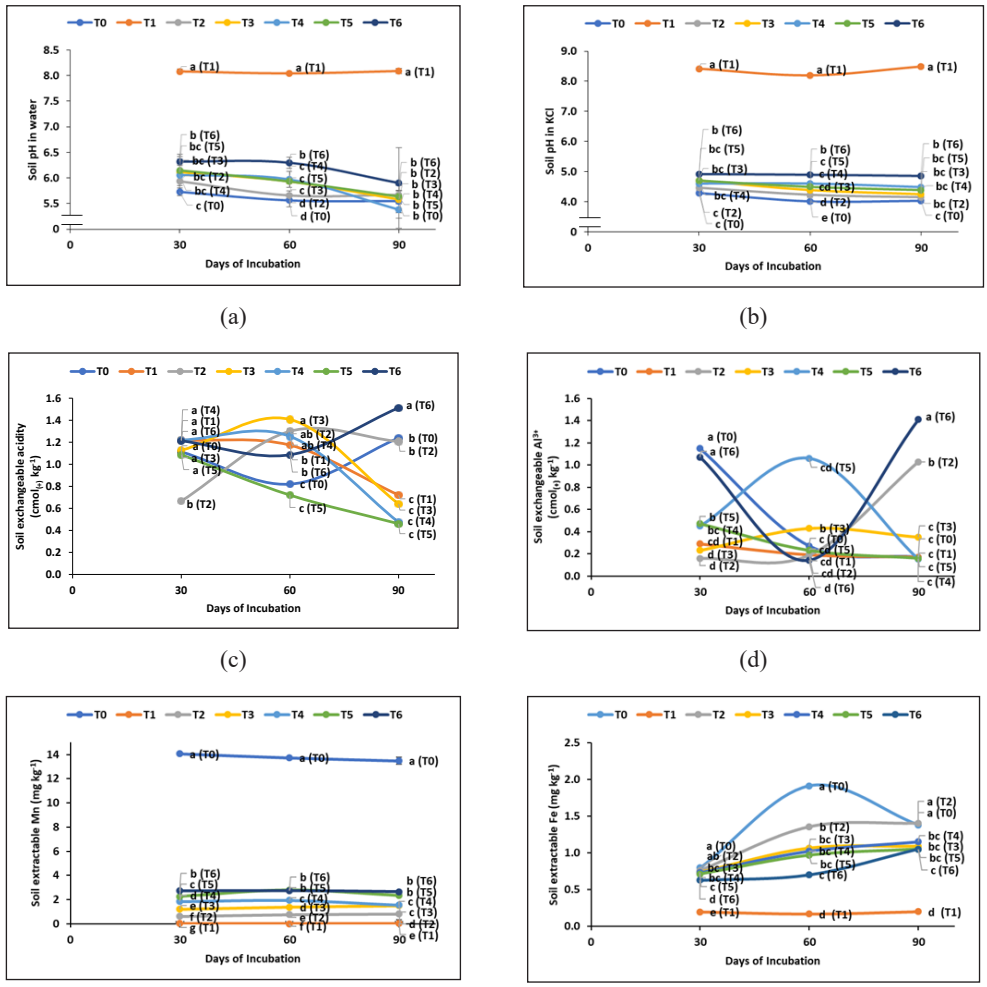


Figure 4. Soil (a) pH in water, (b) pH in potassium chloride, (c) exchangeable acidity, (d) exchangeable aluminium, (e) extractable manganese, and (f) extractable iron in relation to co-application of silico-manganese slag and chemical fertilisers at 30, 60, and 90 days of incubation. Different letters at each sampling time indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF)

Table 5
Effects of sampling time on soil pH, exchangeable acidity and aluminium, and extractable manganese and iron

Soil Chemical Properties	Treatment	Days of Incubation		
		30	60	90
pH in water	T2	5.94a±0.04	5.66b±0.05	5.67b±0.05
	T3	6.12a±0.20	5.93a±0.03	5.58a±0.15
	T4	6.06a±0.03	5.97a±0.09	5.38b±0.21
	T5	6.15a±0.06	5.93ab±0.01	5.64b±0.16
	T6	6.32a±0.03	6.30a±0.06	5.90a±0.40
pH in KCl	T2	4.47a±0.04	4.23b±0.02	4.15b±0.02
	T3	4.70a±0.22	4.39a±0.03	4.25a±0.07
	T4	4.60a±0.02	4.49ab±0.06	4.17b±0.12
	T5	4.70a±0.03	4.49ab±0.01	4.39b±0.09
	T6	4.92a±0.03	4.90a±0.00	4.86a±0.40
Exch. acidity ($cmol_{(+)}\ kg^{-1}$)	T2	0.67b±0.05	1.30a±0.03	1.21a±0.04
	T3	1.13a±0.12	1.41a±0.04	0.64b±0.06
	T4	1.22a±0.05	1.25a±0.05	0.47b±0.11
	T5	1.09a±0.03	0.72b±0.08	0.46c±0.02
	T6	1.21b±0.04	1.09b±0.03	1.51a±0.03
Exch. Al^{3+} ($cmol_{(+)}\ kg^{-1}$)	T2	0.16b±0.00	0.19b±0.03	1.03a±0.05
	T3	0.23a±0.01	0.43a±0.01	0.35a±0.13
	T4	0.45b±0.05	1.06a±0.05	0.15c±0.01
	T5	0.47a±0.07	0.23b±0.02	0.16b±0.01
	T6	1.07b±0.02	0.14c±0.02	1.41a±0.06
Extractable Mn ($mg\ kg^{-1}$)	T2	0.61b±0.01	0.76a±0.02	0.82a±0.01
	T3	1.21b±0.01	1.39a±0.04	1.49a±0.05
	T4	1.84a±0.04	1.94a±0.13	1.55a±0.09
	T5	2.26a±0.06	2.79b±0.03	2.33a±0.03
	T6	2.73a±0.04	2.76a±0.14	2.66a±0.15
Extractable Fe ($mg\ kg^{-1}$)	T2	0.76c±0.01	1.36b±0.01	1.40a±0.01
	T3	0.74c±0.01	1.07b±0.03	1.09a±0.03
	T4	0.73c±0.01	1.03b±0.01	1.15a±0.01
	T5	0.71c±0.03	0.97b±0.02	1.05a±0.03
	T6	0.63c±0.01	0.70b±0.02	0.84a±0.08

Note. Different letters within a row indicate significant mean differences using Tukey's HSD test at $p \leqslant 0.05$. T2=Soil + 100% CF; T3=Soil + 100% SiMn slag; T4=Soil + 75% SiMn slag + 25% CF; T5=Soil + 50% SiMn slag + 50% CF; T6=Soil + 25% SiMn slag + 75% CF

Co-Application of Silico-Manganese Slag and Chemical Fertilisers on Soil Exchangeable Ammonium, Available Nitrate and Phosphorus, and Electrical Conductivity

Generally, regardless of sampling time, exchangeable NH_4^+ and available NO_3^- in the soils with chemical fertilisers (especially T2, T4, and T5) were significantly higher compared with those in the soils without any amendments (T0) (Table 6; Figures 5a and 5b). On the contrary, the soils with sole application of SiMn slag (T3) did not significantly increase exchangeable NH_4^+ and available NO_3^- at 90 DAI compared with those at 30 DAI (Table 7). These findings suggest that chemical fertilisers were the main source of supplying nutrients in the Bekenu series. In other words, SiMn slag could only be used as a liming material due to its alkaline nature, whereas chemical fertilisers remain necessary for providing sufficient nutrients for optimum crop growth. Therefore, in further studies, the justification for fixing the application rate of SiMn slag as a liming material should be focused on acid-neutralising capacity, $CaCO_3$ equivalent, target pH, exchangeable acidity neutralisation requirement, or Si/Ca/Mg supply, rather than mass substitution between SiMn slag and CF, as adopted in this soil incubation study. In terms of sampling time, regardless of treatment, the combined use of SiMn slag and CF did not significantly increase the availability of exchangeable NH_4^+ and available NO_3^- over an incubation period of 90 days (Table 7).

Generally, the soils with the amendments (especially T4, T5, and T6) significantly enhanced phosphorus (P) availability compared with soil without any amendments (T0) at 30 and 60 DAI (Table 6 and Figure 5c). The significant increase in P availability could be related to a significant increase in soil pH and a reduction in exchangeable Al^{3+} as well as extractable Mn and Fe. For example, the increased soil pH from the initial pH in KCl of

Table 6
Two-way analysis of variance (ANOVA) results to determine the effects of treatment, sampling time, and interaction of treatment and sampling time on soil exchangeable ammonium, available nitrate and phosphorus, and electrical conductivity

Source of Variations	Treatment	Time	Treatment* Time	Error
Degree of freedom	6	2	12	42
Variables	F value (p-value)			
Exch. NH_4^+ (mg kg^{-1})	20.97* (<0.0001)	23.96* (<0.0001)	4.80* (<0.0001)	-
Avail. NO_3^- (mg kg^{-1})	31.03* (<0.0001)	31.75* (<0.0001)	7.45* (<0.0001)	-
Avail. P (mg kg^{-1})	25.37* (<0.0001)	5.54* (0.0073)	3.89* (0.0005)	-
EC (µS cm^{-1})	14.79* (<0.0001)	30.89* (<0.0001)	3.09* (0.0033)	-

Note. *indicates significant effects at $p \leq 0.05$

Table 7
Effects of sampling time on soil exchangeable ammonium, available nitrate and phosphorus, and electrical conductivity

Soil Chemical Properties	Treatment	Days of Incubation		
		30	60	90
Exchangeable NH_4^+ (mg kg^{-1})	T2	184.47a±15.18	165.79b±22.22	140.10b±16.18
	T3	184.47a±66.23	191.47a±58.05	59.31a±7.65
	T4	241.44a±11.56	242.84a±24.71	52.30b±14.50
	T5	270.39a±55.36	146.17ab±24.34	58.84b±5.05
	T6	35.03b±4.50	154.11a±16.16	10.74b±1.68
Available NO_3^- (mg kg^{-1})	T2	125.16b±32.20	290.47a±37.25	214.82ab±20.36
	T3	196.14a±57.37	153.18a±53.63	54.17a±2.04
	T4	232.57a±23.20	149.44ab±12.36	81.26b±20.65
	T5	205.01a±77.08	109.28a±9.84	77.99a±7.52
	T6	29.89a±3.74	19.01b±0.57	19.15b±1.24
Available P (mg kg^{-1})	T2	67.57ab±0.69	78.00a±10.64	43.07b±3.76
	T3	62.17ab±9.21	84.07a±8.14	44.70b±3.32
	T4	45.10c±4.60	82.37a±3.33	66.43b±2.40
	T5	117.10a±1.84	90.40a±10.48	52.95b±0.94
	T6	75.17a±4.41	49.33b±5.56	52.83b±3.43
Electrical conductivity (µS cm^{-1})	T2	36.83b±0.67	47.60a±1.01	37.33b±1.62
	T3	34.70a±2.09	58.70a±1.99	60.87a±14.02
	T4	38.60b±0.35	64.03ab±7.48	104.03a±24.68
	T5	40.33a±0.52	56.47a±0.69	64.77a±13.38
	T6	47.57b±1.99	114.77a±9.18	105.87a±19.20

Note. Different letters within a row indicate significant mean differences using Tukey's HSD test at $p \leqslant 0.05$. T2=Soil + 100% CF; T3=Soil + 100% SiMn slag; T4=Soil + 75% SiMn slag + 25% CF; T5=Soil + 50% SiMn slag + 50% CF; T6=Soil + 25% SiMn slag + 75% CF

4.11 to a pH range of 4.15 to 4.89 after applying the SiMn slag in this study significantly reduced exchangeable Al^{3+} as well as Fe and Mn, which could have unlocked the fixed P (Ch'ng et al., 2015; Ch'ng et al., 2016; Ng et al., 2021; Rahman et al., 2014). Reduced exchangeable Al^{3+}, extractable Fe and Mn also decreases P fixation because balanced use of Si increases soil pH to reduce re-sorption of P onto hydrolysed Al, Fe, and Mn minerals (Elisa et al., 2016). Conversely, the lower available P for the soil without any amendments (T0) may be associated with the reaction between P and soluble Al^{3+}, extractable Fe and Mn (Boguta et al., 2019; Ramzani et al., 2016).

In addition, it was speculated that the release of water-soluble Si from SiMn slag in soil solution might have temporarily mobilised the sorbed P, which reacted with inorganic Fe minerals such as ferric oxide. Ng et al. (2024b) and Schaller et al. (2021) reported that an increase in Si availability in soil solution improves P mobilisation. The mechanism

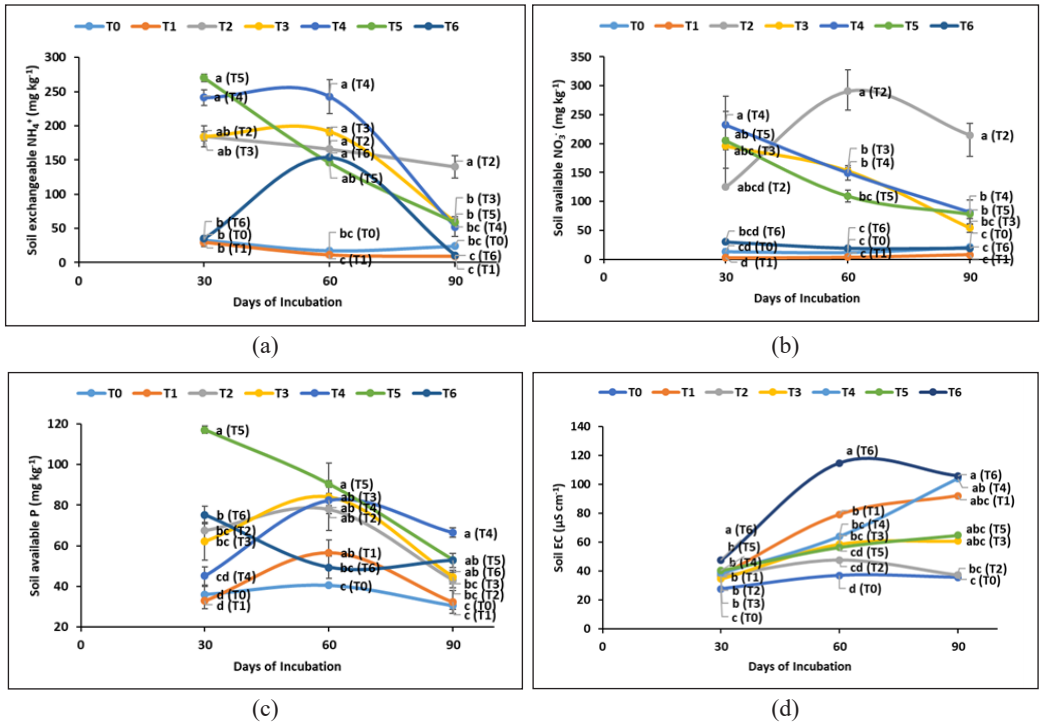


Figure 5. Soil (a) exchangeable ammonium, (b) available nitrate, (c) available phosphorus, and (d) electrical conductivity in relation to co-application of silico-manganese slag and chemical fertilisers at 30, 60, and 90 days of incubation. Different letters at each sampling time indicate significant mean differences using Tukey's HSD test at $p \leq 0.05$. The error bars refer to ± standard error of three replicates

Note. T0 = Soil only; T1 = SiMn slag only; T2 = Soil + 100% CF; T3 = Soil + 100% SiMn slag; T4 = Soil + 75% SiMn slag + 25% CF; T5 = Soil + 50% SiMn slag + 50% CF; T6 = Soil + 25% SiMn slag + 75% CF)

of the release of sorbed P from the inorganic Fe minerals could be related to a stronger bonding affinity of Si to react with soil minerals compared with P (Schaller et al., 2019; Senn et al., 2015). However, similar to exchangeable NH_4^+ and available NO_3^-, regardless of treatment, P availability was significantly reduced at 90 DAI (Table 7), which may be associated with re-adsorption with Al^{3+}, Fe^{3+} and Ca^{2+} over time. Therefore, we recommend that the determination of inorganic P fractionations and sorption studies related to the Si-mediated P mobilisation mechanism should be carried out to further validate the findings of this soil incubation study.

At 30 DAI, EC was significantly increased in the soils with the inclusion of 100% chemical fertilisers (T2), 100% SiMn slag (T3) and combined use of SiMn slag and chemical fertilisers (T4, T5, and T6) compared with soil only (T0) (Table 6; Figure 5d). Notably, at 60 and 90 DAI, there is no significant difference in EC for the soils with the sole application of chemical fertilisers (T2) compared with that of T0. On the contrary, regardless of sampling time, compared with T0, the SiMn slag only (T1) demonstrated significantly

higher EC. In terms of the temporal effects, it was observed that the EC in T2 significantly decreased from 60 DAI to 90 DAI. Conversely, the EC of the soils with combined use of SiMn slag and chemical fertilisers (T4 and T6) showed a significant increase from 30 DAI to 90 DAI (Table 7). These observations suggest that the SiMn slag could be enriched with soluble salts, and its application rate on acidic soils must be optimised. It is recommended to commence a leaching study for elucidating if the SiMn slag could retain nutrients in the highly weathered soils under stimulated raining conditions, whereas future greenhouse and field trials involving maize plants could further validate the soil-plant interaction as well as the liming effects of SiMn slag on maize growth and productivity.

CONCLUSION

Combined use of SiMn slag and chemical fertilisers increased soil pH via the reduction of exchangeable acidity, resulting in reduced exchangeable Al^{3+} as well as extractable Fe and Mn in the Bekenu series over a 90-day incubation period. It was possible that the inclusion of SiMn slag also increased exchangeable NH_4^+, available P, and electrical conductivity in the Bekenu series. The presence of K, Ca, Mg, and Si as elemental composition in the EDX analysis indicates that SiMn slag could be used as a liming agent to mitigate acidity in tropical acidic soils. In this preliminary study, although we intended to replace the reduced amounts of chemical fertilisers by 25% via mass substitution using SiMn slag, the unclear trends of the results indicate that the SiMn slag could be used as a liming amendment, whereas chemical fertilisers remain necessary for supplying nutrient sources. Therefore, rather than the mass substitution approach used in this study, it is recommended that the justification for fixing the application rate of SiMn slag should focus on acid-neutralising capacity, $CaCO_3$ equivalent, target pH, exchangeable acidity neutralisation requirement, or Si/Ca/Mg supply. Moreover, future studies such as sorption, greenhouse, and field trials are necessary to further validate the information regarding the functionality of SiMn slag as an amendment for acidic soils.

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Effect of Nitrogen on Yield and Quality of Forage Sorghum Genotypes in Peninsular Malaysia

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ABSTRACT

Sorghum is a potential source of cellulosic biomass; however, selecting suitable genotypes and providing optimal nutritional supplements are necessary to achieve optimal yield. Proper management practices and fertiliser application will increase the yield and quality of the crop. This study aimed to evaluate the agronomic performance and quality of selected forage sorghum genotypes in response to nitrogen application for cultivation under local climatic conditions. Three forage sorghum genotypes (G1, G2, G3) from different source populations were evaluated for their quality and yield-related components, while grain corn (G4) was utilised for agronomic comparison with the sorghum genotypes only. The experiments were conducted at Field 10, Universiti Putra Malaysia, using a randomised complete block design with a split-plot arrangement, with nitrogen doses as treatments: T1 (120 kg/ha) and T2 (0 kg/ha). Under the influence of nitrogen, G2 (17-39-2-4-3) recorded substantially high stem fresh weights (1248.2 g) and stem diameter (2.87 mm), while G3 (14-9-4-1-2) showed the highest total soluble contents (16.55%) and chlorophyll index (52.46). G2 had the highest levels of neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the stem at T1 (120 kg/ha N), while the leaves of G3 had higher NDF values under the influence of nitrogen. The study identifies G2 and G3 as potential forage sorghum genotypes that generate good yield and quality under nitrogen application. Nevertheless, the study was conducted under limited nitrogen

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regimes; therefore, an extensive field evaluation is recommended for determining optimal yields under varied nitrogen levels.

Keywords: Chlorophyll, NDF, physiology, sorghum, TSS

INTRODUCTION

Sorghum (Sorghum bicolour (L.) Moench) is the fifth most important cereal crop worldwide. It originated from North Africa and is cultivated across continents in countries including China, India, and Mexico (Venkateswaran et al., 2019). It can be cultivated in challenging environments with significantly high productivity. Sorghum has been widely introduced as a forage crop in numerous tropical and subtropical regions across the globe (Dicko et al., 2006; Dexuan et al., 2022). It has become a staple food source in the semi-arid tropics of Asia and Africa and is also a preferred fibre food for millions of people worldwide (Hariprasanna, 2015; Mohammed et al., 2019). In Southeast Asia, Thailand is a major sorghum producer, with an annual production of more than 50,000 tons, while China and India are the largest sorghum producers on the Asian continent (Taylor, 2019).

Sorghum can be used for feed and fodder, and it is chosen as a potential animal feed because of its high cellulosic and hemicellulose content (Bartzialis et al., 2020; Hao et al., 2014). Even though water and good soil conditions have been reported among the main factors of sorghum yield limitation, as this crop is typically grown under rain-fed conditions (Bakari et al., 2023). However, for many years, sorghum has also been grown extensively in marginal soils where nitrogen deficiency affects the productivity of the crop, as it is the major component of plant nutrition. The tuning of the nitrogen component of the soil will apparently increase the biomass of sorghum (Franke et al., 2018; Wortmann et al., 2007). Effective fertiliser management practices can enhance growth rate, biomass, plant height, and leaf area index. Suitable genotype selection along with adequate fertilisation application will improve the biomass yield of sorghum. This will help reduce input costs and prevent the loss of available nutrients (Baligar et al., 2001; Silva et al., 2024).

Nutrient efficiency is the most important factor for breeding new cultivars. Nitrogen has been effectively found to affect the growth and productivity of sorghum and other grasses (Rihacek et al., 2020). Sorghum has a very minimal fertiliser requirement, and efficient nitrogen (N) utilisation in grass production is highly recommended to reduce production costs (Afzal et al., 2012; Tasie & Gebreyes, 2020). The cell wall is composed of key traits such as protein content, neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL), which are all quality indicators for forage applications (Yucel & Erkan, 2020). These components are directly obtained from the nutrients supplemented to the crop, which enhances the quality of the sorghum-based animal feed (Oktem et al., 2021). Protein is one of the most important components of sorghum-based feed, which is rich in amino acids and N. The nitrogen content of the feed samples is used to calculate

the total protein content of the forage (Tasie & Gebreyes, 2020). The type and quality of forage are distinguished from one another based on cell wall materials. The nutritional value of forage is also determined by cell wall composition (Rivai et al., 2021).

The amount of digestible forage for the animals is calculated using NDF and ADF values. The insoluble portion of forage tissue is mainly composed of NDF, which further contains hemicellulose, cellulose, and lignin components of the plant cell wall. ADF is part of the feed that is slightly indigestible under acidic conditions, as it contains cellulose, lignin, and other indigestible components. NDF and ADF are good indicators of feed quality and plant maturity within a specific diet. Better estimation of fodder consumption and diets can be designed using NDF and ADF values (Harper & McNeill, 2015; Singh et al., 2018). Forage plants require various nutrients to grow, and different forage plants have diverse nutrient requirements. Irregular and insufficient nitrogen supplementation usually restricts plant productivity and affects the feed crop quality (Alley, 2001; Sayre et al., 2023). Nitrogen is a scarce nutrient in most tropical soils; therefore, sufficient nitrogen administration showed a great impact on the crops when applied in local cultivation practices (Amin, 2011; Arshad et al, 2020). Monitoring the nitrogen supplementation efficiency of crops can reduce production costs while maintaining environmental quality. Heavy use of nitrogenous fertilisers can result in negative environmental impacts, even though the crop shows an increase in yield (Omara et al., 2019).

Malaysia has an equatorial climate, with an average annual rainfall of 250 centimetres. Heavy rainfall can lead to a significant loss of nutrients in the soil, rendering key nutrients unavailable. Additionally, the nitrogen requirements can vary depending on specific crops (Mustaffer et al., 2024). Sorghum holds potential as a crop for forage production, particularly if appropriate sustainable agricultural practices are employed. It is essential to identify the most effective agronomic practices and tailor them to achieve productive forage cultivation. This study was conducted to assess the yield potential and quality of various forage sorghum genotypes under differing nitrogen regimes in Malaysia. Since sorghum cultivation is still in its nascent stages in the country, it is crucial to identify the necessary agronomic practices to optimise sustainable production in local conditions.

MATERIALS AND METHODS

Experimental Location and Planting Material

This study was conducted at Ladang 10, Faculty of Agriculture, Universiti Putra Malaysia (UPM). The land was ploughed and harrowed twice to a depth of 30 cm using a tractor-mounted moldboard plough. Sorghum seeds of three genotypes, including G1 (21-107-1-3-3), G2 (17-39-2-4-3), and G3 (14-9-4-1-2), were obtained from the Agrobank Faculty of Agriculture, UPM, Malaysia. A grain corn genotype was acquired from Famox Company (Kedah, Malaysia) and was designated as G4 in the study for comparative agronomic analysis only. The field experiment started from August to December 2020.

Seeds were sown in germination trays for 7-10 days before being transplanted to the experimental plot.

Experimental Design

The experimental design applied was a split-plot in a randomised complete block design, with three replications. The first factor was the nitrogen rate, which served as the main plot. It had two levels: 120 kg/ha of nitrogen fertiliser (T1) and 0 kg/ha of nitrogen application (T2). The second factor was genotypes, including three sorghum genotypes and one grain corn, assigned as the subplots. The distance between blocks was 2.5 m, the inter-row spacing was 0.7 m, and the interplant spacing was 0.3 m between plants (Chakravarthi et al., 2017; Naharudin et al., 2021).

Data Collection

Agronomic Parameters

The agronomic parameters, such as days to flowering, were observed when the plant reached the desired stage. Plant height was measured from the ground to the collar of the last leaf, while the number of leaves was counted manually. Relative chlorophyll content was measured from the second-to-last opened leaf using a SPAD meter (Konica Minolta SPAD-502Plus). Stem diameter was recorded 15 cm from the ground using a digital Vernier calliper (Mitutoyo), biomass yield, including the fresh weight of leaves and stem, using an electronic balance, and total soluble content was measured using a digital refractometer post-harvest (Chakravarthi et al., 2017; Farahat et al., 2020; Naharudin et al., 2021).

Chemical Analysis of Feed Quality Traits

Feed quality analysis was conducted for sorghum genotypes only. Composites of stem and leaf samples of each genotype were dried in an oven at 70 ºC for 48 hours and ground using a bench-scale Retsch SM2000 (1 mm screen) knife mill. Samples were then analysed for neutral detergent fibre and acid detergent fibre with the Fibertec System, taking their fresh and dried weights into account (Chakravarthi et al., 2017; Van Soest, 1963). Crude protein was determined by the Kjedahl method in which the resulting nitrogen content of the sample are multiplied values are then multiplied by 6.25 to get the crude protein content (Association of Official Analytical Chemists [AOAC], 1990).

Statistical Analysis

A split-plot ANOVA was used to analyse the effects of nitrogen rate, genotypes, and their interactions on all measured variables. Mean comparison using the LSD method was done to determine the best nitrogen rate and genotype ($p<0.05$).

RESULTS AND DISCUSSION

Growth and Yield Performance of Sorghum Genotypes

Nitrogen application had a significant effect on the phenology and growth of genotypes ($p < 0.05$). Different genotypes exhibited varying days to reach flowering; however, G1 under T1 had the earliest flowering at 63 days among sorghum genotypes under nitrogen treatment. (Figure 1A). There was a significant difference in the average stem diameter between the two treatments, specifically 2.47 cm and 2.12 cm, respectively. Under the influence of 120 kg/ha nitrogen, the highest stem diameter of 2.87 cm was shown by G2, followed by G3 (2.487 cm) (Figure 1B). A significant difference in plant height was also observed in the experimental groups treated with nitrogen and those without nitrogen treatment. The average plant height under the influence of nitrogen was 278.59 cm, whereas under no nitrogen treatment, it was 228.66 cm. Among the sorghum lines, G1 showed the highest plant height (320.7 cm), followed by G3 (319.7 cm) and G2 (302.5 cm). Grain corn showed the lowest response to nitrogen and was only 172 cm high (Figure 1C). Nitrogen did not have any significant effect on the number of leaves, while genotypic effects were significant ($p<0.05$). G2 has the highest number of leaves (13.66 leaves), while the lowest number belongs to the grain corn (11.33 leaves) (Figure 1D).

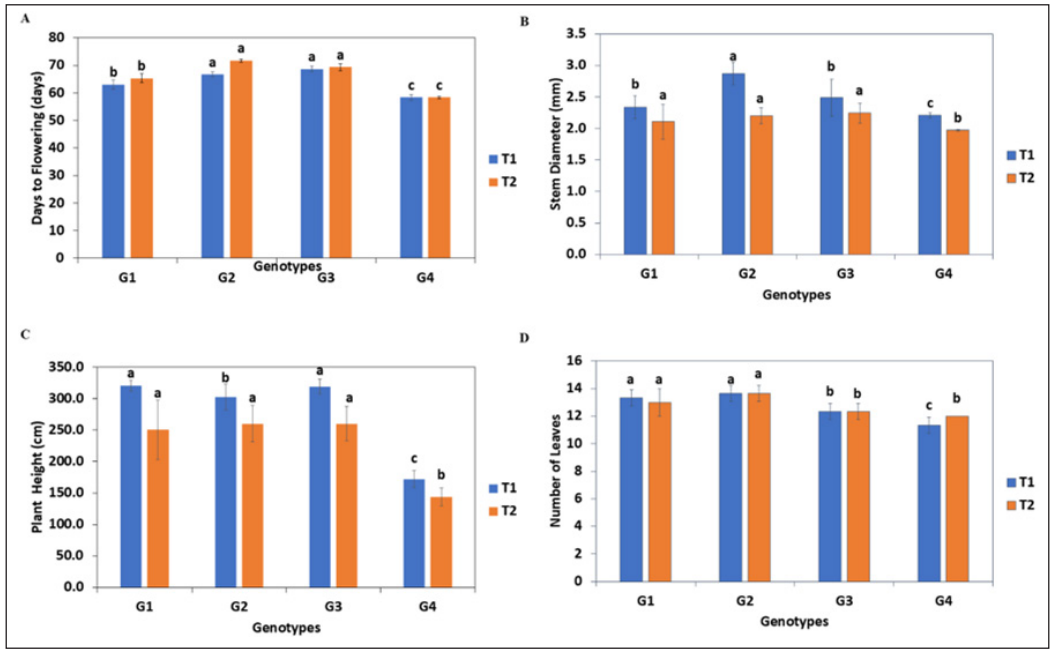


Figure 1. Effect of nitrogen treatments on different characteristics of genotypes: (a) days to flowering (days), (b) stem diameter (mm), (c) plant height (cm), and (d) number of leaves. Small letters show the significant differences ($p<0.05$) among genotypes within the same treatment groups according to the LSD test
Note. Error bars represent standard deviations among the replicates

Nitrogen application had a significant effect on the fresh weight of stems and leaves ($p < 0.05$). G2 recorded the highest fresh weight of stems, 1248.2 g, followed by G3 with a fresh weight of 995.5 g under nitrogen supplementation, while G1 recorded 963.4 g within the same treatment (Figure 2A). The highest average fresh weight of the leaves under nitrogen treatment was recorded by G1 (140 g), followed by G2 (135.8 g). The average fresh weight of leaves without nitrogen treatment was 67.7 g, where G3 showed the highest fresh weight, 80.8 g, followed by G2 with a fresh weight of leaves of 74.6.5 g (Figure 2B). The stem is a vital component of plant architecture that gives structural support, allows transportation of water and nutrients throughout the plant (Javed et al., 2022). Nitrogen is an integral part of amino acids, proteins, and enzymes that are required for cell division, elongation, and differentiation (Ali et al., 2021). When nitrogen is readily available, it facilitates the protein-building process that supports cell growth and development (Anas et al., 2020). This contributes to stem elongation and diameter, and also enables the plant to gain a larger height and accumulate greater biomass. The biomass of sorghum increased upon nitrogen application (Holman et al., 2019).

Total Soluble Solid Content

Nitrogen application has a significant effect on the total soluble content of genotypes ($p < 0.05$). G3 had the maximal total soluble solid content (16.5%), while grain corn had the lowest total soluble solid content (4.3%) at T1(Figure 2C). The difference in total soluble solids between G3 and grain maize points to a considerable variation in the composition or accumulation of sugars. The total soluble solids are the weight percent of water-soluble solids in the stem, and it is widely used as an approximation of sugar content due to their significant linear correlation with total sugar content (Sen et al., 2019). The value of sugar content in sorghum can be enhanced to use it as a feedstock (Jebril et al., 2021). Nitrogen is involved in enhancing photosynthetic activity, thereby producing more assimilates, which can enhance the synthesis of carbohydrates. Therefore, the higher sugar content in the stem under the influence of nitrogen is attributed to enhanced carbohydrate production. The increased value of total soluble solids in sorghum is a key trait for quality assessment and also indicates a sweeter genotype with better flavour and higher nutritional value (Zaituniguli et al., 2021).

Chlorophyll Index

Nitrogen significantly affected the chlorophyll index of the genotypes ($p < 0.05$). G3 exhibited the highest chlorophyll index in T1 (52.46) and T2 (42.22), while G1 was the lowest chlorophyll-producing genotype in T1 (44.09) and T2 (29.56) (Figure 2D). Nitrogen is an important element for plant metabolism. It is an essential part of the chlorophyll structure in leaves necessary for photosynthesis. Nitrogen increases the chlorophyll index of the plant under optimum conditions (Buah & Mwinkaara, 2009; Dembele et al., 2021).

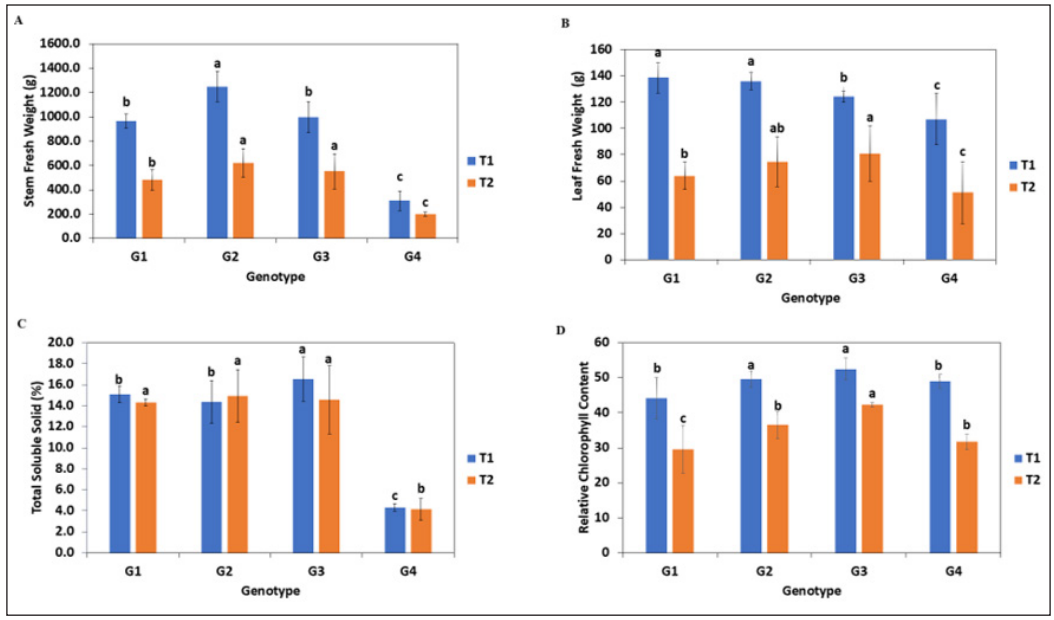


Figure 2. Effect of nitrogen supplementation on different characteristics of genotypes: (a) stem fresh weight (g), (b) leaf fresh weight (g), (c) total soluble contents (%), (d) chlorophyll content. Small letters show the significant differences ($p<0.05$) among genotypes within the same treatment groups according to the LSD test
Note: Error bars represent standard deviations among the replicates

Although grain corn (G4) was included as a comparative genotype for agronomic evaluation, its performance was generally intermediate relative to the sorghum genotypes across most measured traits. Therefore, the discussion focused more on the contrasting responses of the sorghum genotypes, particularly those showing superior agronomic and forage quality characteristics under the study conditions.

Feed Quality Parameters

Crude Protein Content

The effect of nitrogen on crude protein content in sorghum stem and leaf was highly significant ($p < 0.05$). Among the observed genotypes, G2 produced the highest crude protein level of 4.24% under T1 and 2.14% at T2 in the stem. However, G1 produced the lowest crude protein of 2.08% under 120 kg/ha nitrogen supplementation, and 1.75 % at no nitrogen application (Figure 3A). The crude protein in leaf ranged from 11.1 to 7.64% across treatments, with G2 producing the highest in T1 (11.1 %) and G1 recording the maximum values in T2 (7.74 %) (Figure 3B). The crude protein contents of leaf and stem in G2 were the highest in nitrogen application treatments. This also pertains to the highest nitrogen efficiency of G2. The crude protein yield decreased significantly with a

reduction in nitrogen doses across all sorghum genotypes. Similarly, Mut et al. (2017) also reported a quadratic response in crude protein content to increasing nitrogen rates across all tested samples. Rihacek et al. (2020) reported similar crude protein ranges of 11.1 to 16.5% while studying the nitrogen efficiency of sorghum genotypes. Likewise, Xu et al. (2021) and Damian et al. (2017) observed that crude protein yield rose significantly with increasing nitrogen doses across all maize and sorghum crops. The crude protein content in leaves observed in the current study was higher than the minimum recommended by Van Soest (2018) for optimal rumen fermentation. Therefore, the evaluated genotypes can be recommended for further selection of high-quality forage. These results were in line with the findings of Toum et al. (2018), who also reported that high nitrogen efficiency increased plant crude protein content. This can possibly be explained by the fact that nitrogen is frequently involved in protein synthesis (Chen et al., 1994; Singh et al., 2018)

Neutral Detergent Fibre Content

The nitrogen application had a significant effect on the stem and leaf neutral detergent fibre content of sorghum genotypes ($p < 0.05$). The fertilised forage had a higher mean NDF concentration in the stem (61.3%) compared with the non-fertilised ones (59.08%). The study also observed that stem NDF content varied among the genotypes as well. G2 produced the highest NDF content with 62.77% in T1, while G3 recorded the lowest (58.03%) (Figure 3C). This result is related to the high stem girth of G2, which raises the amount of cell-wall material in forage. This result aligns with the findings of Carpici et al. (2010), who reported a correlation between forage NDF content and stem diameter. Collectively, these results suggest that increasing plant stem diameter could play a role in elevating NDF content (Sher et al., 2016). Although an increased amount of fibre in forage reduces digestibility. Highly digestible fibre enables greater dry matter intake in animals whose intake is limited by physical fill (Hoffman et al., 2001). Eltelib and Eltom (2006) reported similar results that increased nitrogen concentration enhances the fibre quantity of sorghum. In contrast, Delevatti et al. (2019) reported that an increase in nitrogen levels decreases the NDF concentration. It is worth noting that the high NDF content of the forage is still a dominant factor in determining overall forage quality.

Leaves of sorghum shared contrasting results as compared to the stem, where the mean NDF value for T2 (68.85 %) was higher than T1 (65.95 %), suggesting that nitrogen application did not increase the NDF content in leaves. G3 produced the highest NDF content at T1 (67.22%) and T2 (70.81%), whereas the lowest NDF values in leaves were recorded by G2 (63.9%) at T1 and G1 (66.8%) at T2 (Figure 3D). Nitrogen application promotes protein and chlorophyll content, resulting in less fibre content in leaf tissues (Carpici et al., 2010). Similar findings were reported by Bélanger & McQueen (1999), where the leaf NDF concentration decreased with increasing rates of N fertilisation.

Additionally, the range of NDF found in this study was higher than the National Research Council's dairy cow diet recommendation, affirming their utility as animal feed (Mohammed et al., 2019).

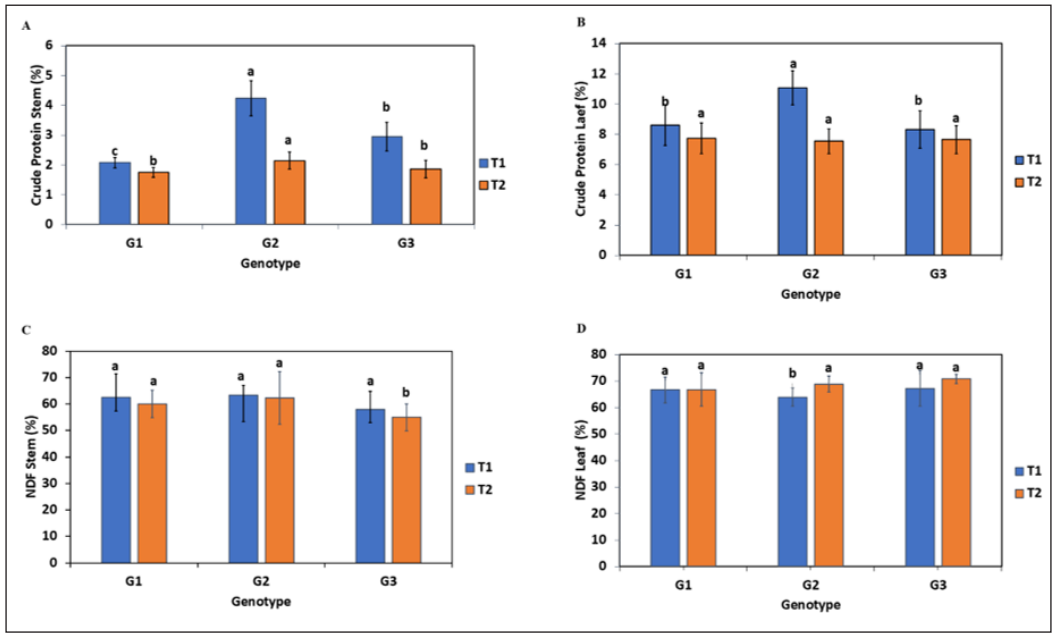


Figure 3. Effect of nitrogen feed on quality parameters of various sorghum genotypes: (a) crude protein in stem (%), (b) crude protein in leaf (%), (c) neutral detergent fibre in stem (%), (d) neutral detergent fibre in leaves (%). Small letters show the significant differences ($p<0.05$) among genotypes within the same treatment groups according to the LSD test

Note. Error bars represent standard deviations among the replicates

Acid Detergent Fibre Content

Nitrogen application significantly affected the ADF content in the stems and leaves of sorghum genotypes, while genotypic differences were observed. The highest ADF content in the stem was observed in G2 (40.81%) at T1 and T2 (39.57%), while G3 produced the lowest values across treatments (Figure 4A). This was probably corresponding to the fact that G2 showed the highest stem diameter. Thus, it contained the highest proportion of cell walls. Cell wall degradability was highly correlated with chemical composition in plants (He et al., 2020). Leaves of G1 produced the highest ADF content of 33.83%, followed by G2 with 33.03% at T1, while G3 recorded the maximum values at T2 (36.21%), and the lowest was generated by G1 (35.09%) within the same treatment (Figure 4B). Johnson et al. (2001) reported similar findings, indicating that the application of nitrogen had a minimal effect on the ADF concentration of plants. This trend is in disagreement with the findings

by Bélanger & Gastal (2000), which indicate that the increase in nitrogen has significantly affected the stem ADF concentration of the forage.

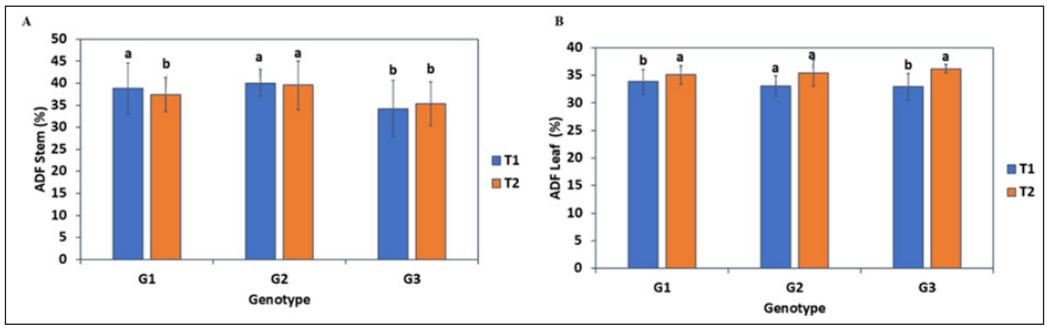


Figure 4. Effect of nitrogen on different characteristics of sorghum genotypes, (a) acid detergent fibre in stem (%), (b) acid detergent fibre in leaves (%). Small letters show the significant differences ($p<0.05$) among genotypes within the same treatment groups according to the LSD test
Note. Error bars represent standard deviations among the replicates

CONCLUSION

Among all sorghum genotypes studied, G2 showed higher biomass yield, stem diameter, and the highest crude protein values in the stem under the influence of nitrogen. Whereas G3 recorded the maximum total soluble solids and relative chlorophyll content, and also generated the maximum NDF content in leaves in response to nitrogen application. The study concludes by identifying G2 and G3 as potential forage sorghum genotypes that yield well and maintain quality in response to nitrogen application and are therefore suitable for development as nitrogen-efficient sorghum genotypes. The experiment was preliminary and involved a dose-specific trial with limited observations; therefore, extensive field evaluations are required to assess performance under varied nitrogen regimes.

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CONFLICTS OF INTEREST

The authors do not have any conflict of interest related to the design, data collection, analyses, interpretation or writing of the manuscript.

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Brucella spp. Surveillance in Wild Long-Tailed Macaques (*Macaca fascicularis*) in Selangor, Malaysia

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ABSTRACT

Brucellosis is a zoonotic disease that is caused by the intracellular Gram-negative coccobacillus *Brucella* spp., which threatens public health and local socio-economics, with wild and captive non-human primates known to be susceptible to several *Brucella* biovars. In Malaysia, wild long-tailed macaques (*Macaca fascicularis*) frequently inhabit areas near human settlements, increasing human-wildlife conflict and potential disease spillover risk. This study aimed to determine the seroprevalence of anti-*Brucella* antibodies and the presence of *Brucella* antigens in wild long-tailed macaques from selected areas in Selangor, Malaysia. 100 fresh carcasses of the wild long-tailed macaques were retrieved from the Department of Wildlife and National Parks of Peninsular Malaysia (PERHILITAN), and pathological abnormalities were observed in several organs, including the reproductive, hepatosplenic, and lymphatic organs, during necropsy. Serum samples (n=100) were screened using the Rose Bengal Plate Test (RBPT), and reactive samples were confirmed by the Complement Fixation Test (CFT). Reproductive tissues (ovaries, uteri, placentas, testes) were collected for DNA extraction and analysed by qPCR targeting *Brucella* spp. genes. RBPT screening showed 1% positivity (1/100, 95% CI: 0.37%-1.63%), but all sera tested negative by the confirmatory CFT. qPCR analysis of all 100 reproductive tissue samples yielded no amplification of the targeted *Brucella* gene, indicating a 0% antigen presence (0/100, 95% CI: 0-0.627%). Consequently, the overall prevalence of *Brucella* antibodies or antigens in

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wild long-tailed macaques in the study areas ranged from 0% to 1.67% (95% CI). These findings suggest that exposure to *Brucella* spp. and active infection range from very low to non-existent in these wild macaque populations. Nevertheless, continued surveillance is warranted given the zoonotic potential and the proximity of the wildlife population to human settlements.

Keywords: Brucella spp., CFT, human-wildlife conflict, *Macaca fascicularis*, qPCR, RBPT

INTRODUCTION

This bacterial disease was first discovered in the 1850s, plaguing the citizens of Malta Island, located in the Mediterranean Sea. The newly emerging disease was initially named Malta fever before being discovered by a British army surgeon, David Bruce, and then named brucellosis (Vassallo, 1992). Humans, livestock, wildlife, and marine animals are known to be susceptible to brucellosis (Wyatt, 2013). Typically, this zoonosis is transmitted between farmed animals, where it is also transmissible to humans and wildlife through direct contact, consumption of raw milk, dairy products, and meat; exposure to infected animals and their bodily secretions; and exposure to contaminated aerosol and bacteria cultures can cause infection via mucous membranes and conjunctiva (Küplülü et al., 2004; Piao et al., 2020; Pereira et al., 2020; Sack et al., 2018).

The clinical manifestations of brucellosis in animals primarily involve reproductive complications such as abortion, orchitis, epididymitis, sterility, and infertility. Alternatively, this disease also induces pathological alterations in various body systems, including the musculoskeletal, cardiovascular, lymphatic, hepatobiliary, renal, and nervous systems (Pradeepkiran et al., 2021). Moreover, Mazlan (2016) detailed the pathological manifestations in animals infected with *Brucella*, including swollen tissue, inflammation, necrosis, petechial haemorrhages, mastitis, endometritis, and cystitis. Additionally, Christopher et al. (2010) have documented that the presence of pathological lesions in brucellosis cases, especially in the reproductive organs and reticuloendothelial systems, has resulted in abortion, as well as enlargement of the liver (hepatomegaly) and spleen (splenomegaly).

On top of that, individuals in direct and frequent contact with infected animals, such as farmers, abattoir workers, and veterinary experts, face an elevated likelihood of contracting *Brucella (Dadar et al., 2022)*. Due to a lack of research and demand, there are currently no commercially available vaccinations for humans against *Brucella*. Hence, the laboratory personnel working on diagnosing the samples from infected individuals and creating the vaccines against these bacterial species are equally at risk of contracting laboratory-acquired brucellosis (Sun et al., 2021).

Research concerning brucellosis zoonotic transmission from wild fauna to humans, particularly non-human primates, is still in its infancy. Interestingly, non-human primates

possess analogous anatomical and physiological traits to their human counterparts, rendering them the most suitable subjects for investigating the epidemiology of human brucellosis and developing vaccines against *Brucella* in humans (Henning et al., 2012). The depletion of animal habitats and natural resources compels wild species to seek refuge and sustenance in human settlements, which offer ample food and shelter for their continued survival (Magouras et al., 2020). This would ultimately escalate the human-wildlife conflict and heighten the likelihood of zoonotic illnesses being transmitted from wildlife to humans when they coexist in close proximity.

The wild long-tailed macaque (*Macaca fascicularis*) is distributed widely across Southeast Asian countries, like Malaysia, Singapore, Thailand, Indonesia, the Philippines, Laos, Vietnam, Cambodia and Bangladesh (Liedigk et al., 2015). This species is one of the arboreal frugivorous non-human primates more commonly responsible for human-wildlife conflicts in Malaysia. Public reports to the Wildlife Department in Peninsular Malaysia indicate that long-tailed macaques are Malaysia's most prevalent non-human primates that instigate conflicts between humans and wildlife (Perhilitan, 2018). Nevertheless, several potential risk of wildlife zoonosis transmission that originating from close interaction with wild macaques can leads to wildlife-human conflict and exposes humans to multitude arrays of diseases such as malaria, tuberculosis, leptospirosis, and meliodosis (Lekko et al., 2021; Saechan et al., 2022).

In order to enhance the efficacy of *Brucella* detection and provide a more precise diagnosis for early illness management, a molecular diagnosis of *Brucella* antigen is strongly advised, in addition to serological findings. Although a bacterial culture is regarded as the gold standard to identify bacteria and provides stronger assurance for true positive results, given its prevalence as a laboratory-acquired infection, brucellosis poses potential risks to laboratory personnel due to its lengthy incubation period, low positivity rate, and possible violation of biosafety protocols (Traxler et al., 2013; Song et al., 2021). Moreover, Yagupsky et al. (2019) reported that bacterial isolation and *Brucella* strain identification also require extended time and effort, which may compromise the diagnosis process and the epidemiological surveillance for mitigating outbreaks. Hence, molecular assays were employed to highlight the amplification of nucleic acids to detect the *Brucella* antigen, facilitating prompt identification and verification of the bacterial species.

Brucella antigen detection through molecular qPCR has a high specificity of 100% and a high sensitivity of 93.14% (Che et al., 2019). When extracting samples for molecular diagnosis, different kinds of samples can be extracted, such as bacterial cultures, physiological fluids (e.g., blood, milk, and semen), and tissues (e.g., placenta, aborted foetuses, reproductive organs, lungs, and lymph nodes) (Amin et al., 2001; Gallien et al., 1998; Ilhan et al., 2008; Manivannan et al., 2021; Nyarku et al., 2020; Yu & Nielsen, 2010). Then, multiplex real-time PCR is an effective method for quantifying antigen DNA

copies, identifying *Brucella* strains or biovars, and differentiating false-negative results from serological assays, as was found by Dal et al. (2019). Similarly, Che et al. (2019) reported that qPCR detected high *Brucella* DNA loads in some of the negative samples tested by Plate Agglutination Test (PAT) and Serum Agglutination Test (SAT), a detection that demonstrates how molecular tests like PCR are capable of aiding in the diagnosis of early exposure and acute brucellosis. These reports are useful for elucidating the process of transmission of zoonotic diseases, particularly between humans and wildlife (Chisi et al., 2017; Katsiolis et al., 2022).

To date, only one preliminary serological study (Yong, 2017) has assessed *Brucella* exposure in long-tailed macaques in Peninsular Malaysia, using stored sera and RBPT alone without confirmatory testing or molecular detection. No study has combined serological confirmation like CFT with direct antigen detection like qPCR in reproductive tissues, the primary sites of *Brucella* persistence and shedding. This gap is critical because long-tailed macaques in Selangor frequently inhabit densely populated human settlements, where they contact humans, domestic animals, and contaminated environments. Without baseline data on both antibody prevalence and active infection, the zoonotic risk posed by these peri-urban macaques cannot be assessed, and public health authorities lack evidence to guide surveillance or mitigation strategies. Therefore, this study aimed to fill that gap by addressing the following objectives: (1) to screen wild long-tailed macaques from selected areas in Selangor for anti-Brucella antibodies using RBPT and confirm with CFT; (2) to detect *Brucella* spp. DNA in reproductive tissues via qPCR; (3) to estimate the combined prevalence of exposure and infection; and (4) to provide baseline data for zoonotic risk assessment in human-macaque conflict zones.

MATERIALS AND METHODS

Study Design

The study was conducted in Selangor, Peninsular Malaysia, which lies between latitudes 2°35‘ N and 3°60’ N and longitudes 101°14‘ E and 101°46’ E. The region has a tropical rainforest climate with average annual rainfall of 2,500 mm, mean daily temperatures ranging from 27 °C to 32 °C, and relative humidity of 80-85%. The target population was the finite population of wild long-tailed macaques (*Macaca fascicularis*) in Peninsular Malaysia, estimated at 133,403 individuals (Karuppannan et al., 2014). The sample size was calculated using Open-Epi Version 3 with the standard formula for frequency in a finite population as in Equation 1:

$$n = [DEFF \times N \times p(1-p)] / [(d^2/Z^2_{1-\alpha/2} \times (N-1)) + p(1-p)] \quad [1]$$

In this formula, N represents the population size, around 133,403; p is the expected prevalence, which was set at 50% to maximise sample size; d is the desired confidence limit

at 5%, and DEFF is the design effect for cluster surveys, which was set at 1. The minimum required sample size was 95, which was rounded up to 100. A cross-sectional study design was employed. Using convenience sampling, 100 wild long-tailed macaques were obtained from the Malaysia Wildlife Department (Perhilitan) between July 2022 and March 2023.

Post-mortem Examination and Sample Collection

Fresh carcasses were collected from the Perhilitan Selangor district office immediately after chemical euthanasia. All carcasses were transported to the post-mortem laboratory at Universiti Putra Malaysia (UPM) within six hours. A complete necropsy was performed on each carcass. The signalment of each animal was recorded, and any pathological abnormalities or lesions were documented. To minimise the risk of zoonotic transmission, particularly aerosol, wound, and mucous membrane exposure to *Brucella* spp., strict biosafety precautions were followed throughout the necropsy.

Blood was collected from each fresh carcass as soon as possible after arrival. Blood was drawn using three methods: vena cava venipuncture, suborbital vein puncture, and intracardiac venipuncture. All blood was transferred into plain tubes and allowed to clot, after which the serum was separated by centrifugation. Reproductive organs, including the uterus, ovaries, and testes, were harvested. Associated tissues such as amniotic fluid, placenta, and foetus were also collected when available. All tissue samples were stored at -20 °C until further analysis.

Serological and Molecular Detection

Rose Bengal Plate Test (RBPT)

The Rose Bengal Plate Test (RBPT) is a rapid, qualitative serum agglutination assay that detects anti-*Brucella* antibodies (Monlab, 2013). For each serum sample, a drop of Rose Bengal reagent was placed next to a drop of serum on a glass plate. Positive and negative control sera were included in each run. A separate stirrer was used for each sample to mix the reagent with the serum. The plate was then rocked gently for four minutes. The presence of any agglutination or visible clumping was interpreted as a seropositive result (Department of Veterinary Services, 2020).

Complement Fixation Test (CFT)

All sera, regardless of RBPT results, were sent to the Veterinary Research Institute (VRI) in Ipoh, Perak, Malaysia, an accredited diagnostic laboratory, for confirmatory testing. The Complement Fixation Test (CFT) was performed strictly according to the World Organisation for Animal Health (WOAH) (2022). Sera producing a titre of 20 ICFTU/mL (International Complement Fixation Test Units per millilitre) or higher were classified as

positive for anti-*Brucella* antibodies. Samples showing any degree of haemolysis could not be accurately analysed and were therefore recorded as negative. As reported by Legesse et al. (2023), the CFT has 100% specificity but lower sensitivity compared to RBPT or I-ELISA.

Real-Time Polymerase Chain Reaction (RT-PCR)

DNA Extraction. Genital tissue samples such as testes, uteri, ovaries, and placenta from all 100 macaques were subjected to DNA extraction. The specific numbers of each tissue type were: 56 uteri and ovaries, 6 placentas, and 46 testes. Extraction was performed using the DNeasy Blood & Tissue Kit (69,506, Qiagen, Germany) following the manufacturer's instructions. DNA concentration was measured using a TECAN Nanoquant spectrophotometer (Hinić et al., 2008; Saytekin & Ak, 2018).

Primer and Probe Design. Two genetic targets were selected for *Brucella* detection, as shown in Table 1, which were the 16s-23s rDNA intergenic region and the insertion element IS711 (Nyarku et al., 2020; Probert et al., 2004; Zakaria et al., 2019). Primers and probes were designed using NCBI Primer-BLAST, Primer3, Integrated DNA Technologies tools, and OligoAnalyzer. These tools were used to check for primer-dimer and homodimer formation and to ensure target specificity.

Table 1
The probe, forward and reverse primers for the gene targets 16s-23s rDNA and IS711

Gene Target	Taqman		Gene Sequence
16s-23s rDNA	Primer	Forward	GTCGGCCTTGCGAAGCT
		Reverse	GCCCAGATACCGACGCAAA
	Probe		FAM-ATCTGTGGATCGCGTAGTA-MG
IS711	Primer	Forward	GCTTGAAGCTTGCGGACAGT
		Reverse	GGCCTACCGCTGCGAAT
	Probe		FAM-AAGCCAACACCCGGCCATTATGGTBHQ-1

Source: Nyarku et al., 2020; Probert et al., 2004; Zakaria et al., 2019

qPCR Optimisation. Before running the study samples, real-time PCR conditions were optimised. The optimal annealing temperature for both gene targets was determined to be 63 °C. The optimal primer concentration was 0.4 µL per reaction, and the optimal probe concentration was 2 µL per reaction. These values were selected after testing a range of concentrations (primers at 0.8, 0.6, and 0.4 µL; probes at 0.4, 0.3, and 0.2 µL) and temperatures (61 °C to 63 °C).

Standard Curve. A standard curve was generated for each gene target using ten-fold serial dilutions of a positive control (Masjedian Jazi et al., 2017). Each dilution was run in triplicate. Acceptable qPCR efficiency ranges from 90% to 100%, which corresponds to a slope of -3.3 or lower due to a difference of 3.3 cycles between each of the ten-fold dilutions. In this study, the standard curve for 16s-23s rDNA had an R^2 value of 96%, and the curve for IS711 had an R^2 value of 95%. These values confirmed that the qPCR method achieved near-optimal efficiency.

qPCR protocol. All 100 DNA extracts were run in triplicate on a real-time PCR thermal cycler. Each run included negative controls and positive controls, which were a strand of known *Brucella* DNA. A sample was considered positive for *Brucella* antigen if amplification of the target genes, either 16s-23s rDNA or IS711, was detected within the defined threshold cycle (Ct) value established from the standard curve. Samples showing no amplification or Ct values above the threshold were recorded as negative.

RESULTS

After the wild cynomolgus macaque was caught using a metal wall trap and chemically euthanised using barbiturates, all carcasses were collected from the Perhilitan office, where the necropsy was performed in a Biosafety Level (BSL) 2 post-mortem laboratory located at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. The post-mortem examination findings of all wild long-tailed macaque carcasses (N = 100) revealed that 54% of the wild long-tailed macaques (*Macaca fascicularis*) were females, and 6 macaques, or 11% of the 54 females, were discovered to be pregnant. In comparison, 46% were males. Furthermore, other necropsy findings revealed that 2% of the carcasses were emaciated, and ulcerated wounds were found on 2% of the macaques. Besides that, multiple whitish granulomas were found in the gastrointestinal systems, spreading across the stomach until the small intestines in 5% of the samples, which may indicate encysted gastrointestinal endoparasites.

Moreover, the hepatobiliary system demonstrated in Figure 1 had the highest numbers of macroscopic pathological changes (16%), like hepatomegaly at 1%, focal hepatic necrosis at 2%, hepatic granuloma at 5%, hepatic lipidosis at 6%, and hepatic ecchymotic haemorrhage at 2%. Besides that, 8% of the wild long-tailed macaques showed splenomegaly, 1% had a spleen infarction, and 4% had granulomatous lesions on the spleen. Next, the reproductive system (12%) revealed 1% with a vaginal prolapse, 3% with a uterus ecchymotic haemorrhage, 1% with uterus congestion, and 5% with undescended testes, which are indicative of a young age, around less than one year old or juvenile. In comparison, 2% of male macaques showed unilateral testicular hypoplasia. For the renal system, only 1% were discovered to have a unilateral right renomegaly; for the

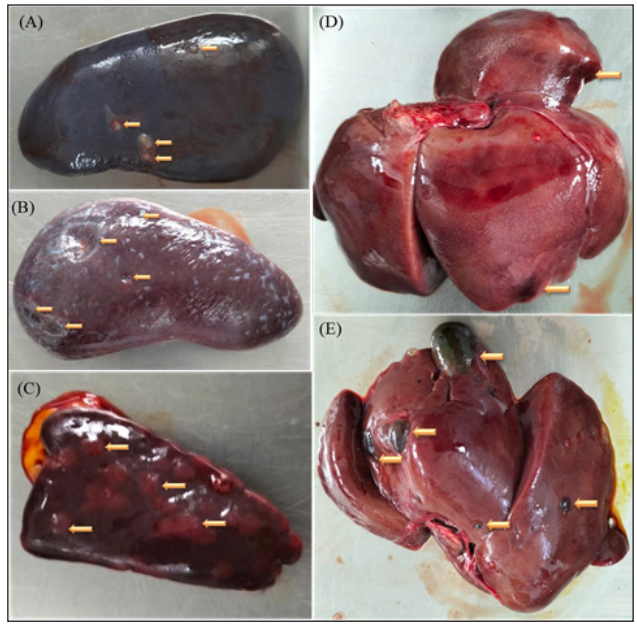


Figure 1. (A) Multiple whitish, raised foci suggestive of granulomatous splenitis (arrows). (B) Multiple depressions and pits of contraction (arrows) were observed on the surface of a spleen with a change in consistency suggestive of splenic infarction. (C) Multiple whitish, raised foci in the parenchyma of a spleen are suggestive of granulomatous splenitis. (D) Presence of a focus of dark red discolouration at the edge of the liver's median lobe and a generalised red discolouration of the liver, suggestive of hepatic haemorrhage and congestion. (E) Multiple cysts of various sizes are embedded in the liver parenchyma as well as on the liver surface (arrows), consistent with multiple hepatic cysts

lymphatic systems, 1% had enlarged submandibular lymph nodes, and 3% had ecchymotic haemorrhage at bilateral inguinal lymph nodes.

Serology

Only one macaque exhibited a positive reaction (1%) against the RBPT through an agglutination reaction observed in Figure 2, indicating the potential presence of antibodies against the *Brucella* spp. antigen in this individual, as shown in Table 2.

Table 2
The Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT) results of free-ranging long-tailed macaques in Selangor

Serological Tests	Reactions	Results	Percentage of Reaction
RBPT	Positive	1	1%
	Negative	99	99%
CFT	Positive	0	0%
	Negative	100	100%

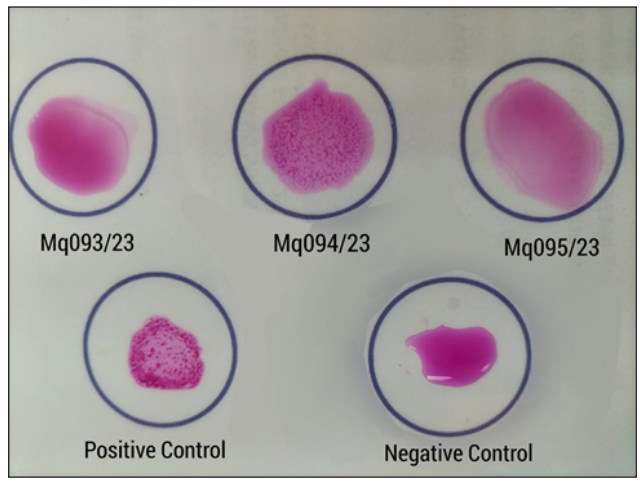


Figure 2. Rose Bengal Plate Test (RBPT) was performed concurrently with positive and negative controls on one hundred sera collected from wild long-tailed macaques

Conversely, the remaining 99% of the wild long-tailed macaques exhibited an adverse response or showed no apparent agglutination of blood cells when exposed to the Rose Bengal stain containing *Brucella* spp. antigen. According to the CFT results, all sera were determined to be negative, including the serum with a positive reaction on the RBPT.

Molecular

The 16s-23s rDNA and IS711 gene targets had threshold cycle (CT) values for the positive control of 24.98 and 24.69, as mentioned in Table 3. Whereas the negative control had CT values of 34.51 for 16s-23s rDNA and 36.77 for IS711 gene targets. Figure 3 revealed that for both the 16s-23s rDNA and IS711 gene targets, 0% of the extracted DNA samples from the related reproductive tissues in the wild long-tailed macaques (*Macaca fascicularis*) had CT values near the cut-off CT values that were established for both IS711 and 16s-23s rDNA, indicating negative results for the Brucella antigen. The qPCR results for all the reproductive tissues would be interpreted as positive if the CT value had been lower than the cut-off value, and if the CT values had exceeded the cut-off points, then it would have been considered a negative result. By simple logistic regression (Logit), the Limits of

Table 3
The CT values for 16s-23s and IS711 to identify the Brucella *spp. gene target from the associated reproductive organs of the cynomolgus macaques*

CT Values Range	16s-23s	IS711
0-5	0%	0%
5-10	0%	0%
10-15	0%	0%
15-20	0%	0%
20-25	20%	5%
25-30	20%	20%
30-35	45%	50%
>35	15%	25%

Detection (LODs) (95% detection rate) for the real-time PCR assays developed in this study and confirmed using a 10-fold serial dilution in triplicate for two different gene targets ranged from 10^6 to 10^{10} copies/µL. As anticipated, all tested genes were successfully identified without any cross-detection, and the amplification signals remained specific to the target gene up to at least cycle 25. The LODs were approximately 4,717 copies per reaction for the target gene 16s-23s rDNA, while there were 3,305 copies per reaction for the IS711 target gene.

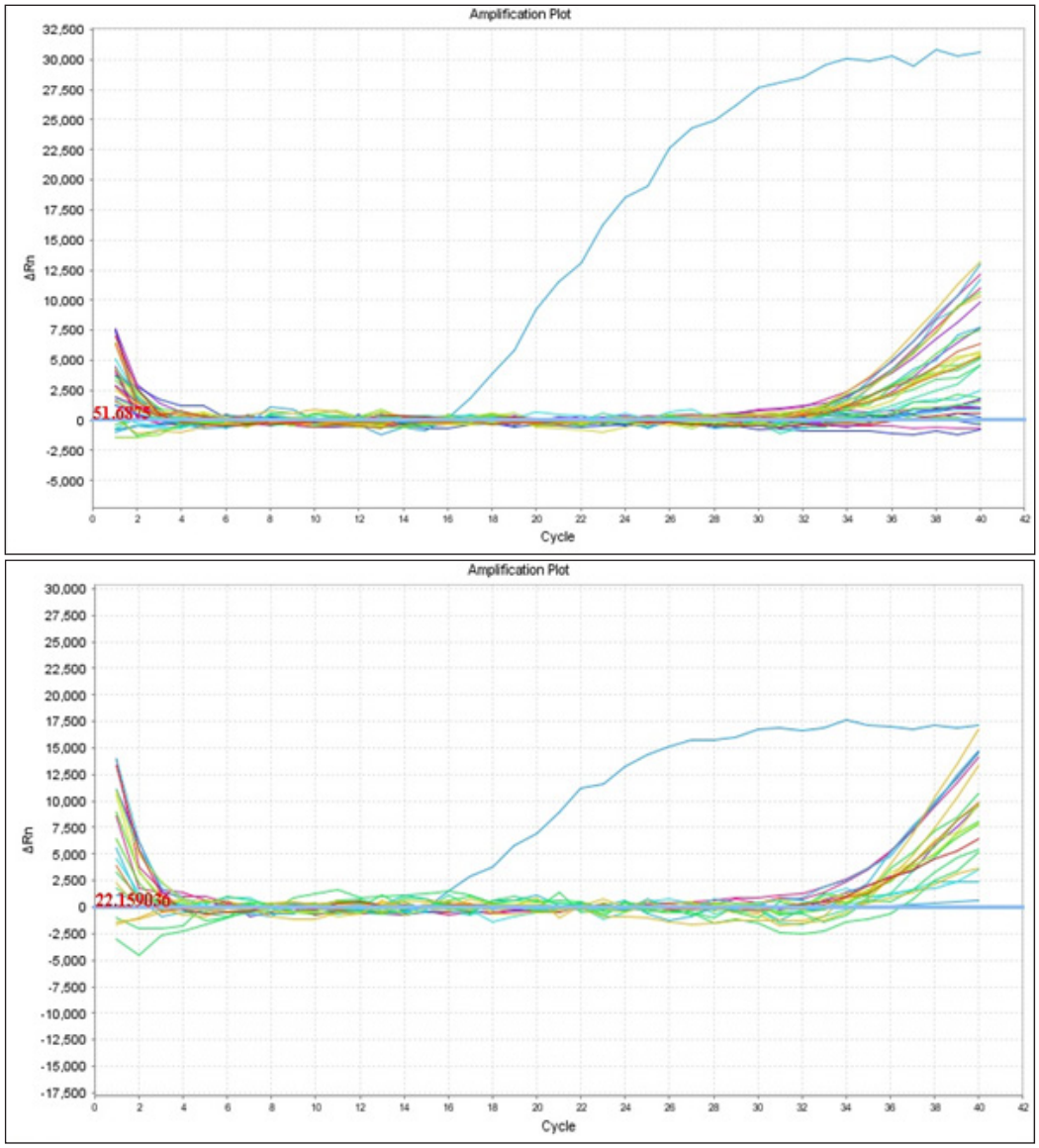


Figure 3. The real-time PCR amplification plot results of the gene sequence to detect *Brucella* spp. antigen. 16s-23s rDNA (upper); IS711 (lower)

DISCUSSION

Brucellosis is a zoonotic infection that affects various species, including non-human primates. Nevertheless, other non-human primate species that were caught from the wild and kept in captivity either through natural infection or through experiment were found to be susceptible to various *Brucella* spp. biovars that are also susceptible to humans (De Mello et al., 1964). Like in certain cases where a new strain of *Brucella* spp. called *B. papionis* was found in Baboons which opens up the possibilities of a new strain of Brucella to occur in other NHP (Huddleson et al., 1929; Schlabritz-Loutsevitch et al., 2009; Whatmore et al., 2014). Interestingly, there are evidence of *B. papionis* that was susceptible to humans trophoblast *in-vitro* where the normal physiological function of the cells were modified (García-Méndez et al., 2019).

This zoonosis is primarily characterised by macroscopic pathological lesions that predominantly target the reproductive system. These lesions can manifest in different clinical forms, including infertility, metritis, retained placenta, abortion, and orchiepididymitis. Furthermore, as highlighted by Mazlan (2016), *Brucella* spp. infection in animals can also lead to additional symptoms, including tissue swelling, inflammation, necrosis, petechial haemorrhages, mastitis, endometritis, and cystitis. In this study conducted on wild long-tailed macaques (*Macaca fascicularis*), 12% of the animals examined were found to have pathological lesions in their reproductive organs, including unilateral testis hypoplasia or undescended testes (1%), uterine ecchymotic haemorrhage (3%), and uterine congestion (1%). These lesions, while consistent with brucellosis, are not pathognomonic and require differentiation from other infectious causes.

Magden et al. (2015) furthered our understanding of differential diagnoses associated with different types of pathological lesions in the reproductive organs, liver, and spleen. Some other infectious diseases have overlapping symptoms with brucellosis, such as leptospirosis, campylobacteriosis, yersiniosis, and tuberculosis, all of which are zoonotic. These diseases can also affect other non-human primates, leading to similar pathological findings in both the acute and chronic stages of testing (Magden et al., 2015; Thayaparan, 2014). Therefore, the presence of reproductive lesions alone cannot confirm *Brucella* infection, and the negative serological and molecular results in this study further support the absence of brucellosis as the cause of those pathologies.

In addition to reproductive system abnormalities, further pathological findings in the hepatobiliary system (16%) included hepatic granulomas in 5% of the cases, hepatomegaly in 1%, hepatic necrosis in 2%, and hepatic haemorrhage in another 2%. Moreover, 13% of splenic abnormalities were observed, with splenomegaly accounting for 8%, splenic infarction making up only 1%, and splenic granulomas accounting for 4%. Then, lymphatic tissues were also affected, affecting the inguinal lymph nodes of 3% of macaques and the submandibular lymph nodes of 1% of macaques. The proximity of the inguinal lymph

nodes to the reproductive organs suggests that there could have been a septic cross-infection between the bloodstream and lymphatic systems. However, as with the reproductive lesions, these hepatosplenic and lymphatic findings are non-specific and were not correlated with any evidence of *Brucella* infection in this study.

The serological testing of 100 wild long-tailed macaques revealed that 99% of blood samples were negative for serum agglutination when exposed to the RBPT with *Brucella* spp. antigen. Consequently, these sera were classified as negative since they showed no detectable antibodies against *Brucella* spp. However, 1% of the macaques' sera tested positive for antibodies, indicating a positive reaction and suggesting that wild long-tailed macaques, similar to rhesus macaques (*Macaca mulatta*), may be susceptible to *Brucella* spp. infection, as has been demonstrated in previous studies involving exposure to contaminated aerosols (Yingst et al., 2010). Nevertheless, all sera were confirmed negative (0%, 95% confidence interval) upon further testing with the CFT. This single RBPT-positive result is therefore most likely a false positive, a known limitation of RBPT due to its lower specificity compared to CFT (Lighter, 1953).

These findings are consistent with a previous serological survey of wildlife in Selangor and Pahang by Zulhisam et al. (2026), which tested 105 archived sera from wild long-tailed macaques (N = 54), wild boars (N = 29), and Asian elephants (N = 22) using RBPT. That study similarly found only one positive result, which came from a wild boar, while all macaque and elephant samples were negative. The authors concluded that the lack of human-livestock-wildlife interface in the study areas likely limits brucellosis transmission cycles, leading to a low prevalence of brucellosis in Malaysian wildlife (Zulhisam et al., 2026). Importantly, that study also emphasised that serological screening results should always be supported with a confirmatory test such as CFT or ELISA, a recommendation that aligns directly with the methodology employed in the present study.

In terms of test performance, the RBPT is known to have higher sensitivity (95.6%) but lower specificity (94.8%) when compared to the CFT, which has higher specificity (96.8%) but lower sensitivity (74.4%) (Teng et al., 2017). As an easy, quick in situ serological screening test, RBPT is always recommended by the WOAH brucellosis protocol but has to be compared with a confirmatory serological test like CFT or ELISA. In contrast, Erdenliğ Gürbilek et al. (2017) reported that CFT, while a reliable test, can yield fewer positive results compared to other serological assays, often due to limitations such as requiring larger serum volumes, being costly, and sometimes providing false-negative outcomes. Despite the limitations, CFT and RBPT were found to have comparable sensitivities (95%) in some studies, as described by Legesse et al. (2023), making them both viable tools for serological tests for antibody detection. The complete concordance between negative CFT results and negative qPCR results in this study reinforces the conclusion that no active or past infection was confirmed.

For this study, molecular detection using real-time PCR (qPCR) was also conducted on the macaques for the purpose of analysing the presence of the *Brucella* spp. antigen. However, no amplification of DNA was observed in any of the 100 macaque samples, indicating the absence of the *Brucella* spp. antigen in reproductive tissue samples, as confirmed by the cycle threshold (CT) values. The failure to detect *Brucella* spp. DNA precludes the ability to accurately quantify *Brucella* spp. in the macaques‘ tissues using qPCR. This finding is consistent with the negative CFT results and strongly suggests that none of the 100 macaques carried an active *Brucella* spp. infection in the reproductive tissues examined.

Seeing as there was a lack of amplification in qPCR results in conjunction with the single positive RBPT result, which was not confirmed by CFT, the RBPT-positive macaque may have previously been exposed to *Brucella* spp., leading to transient antibody development without persistent bacterial colonisation. However, given that the CFT, a more specific test, was negative, the more parsimonious explanation is a false-positive RBPT. The likelihood of detecting the antigen during molecular analysis can be impacted by the selection of the proper tissue samples, namely tissues from reproductive, hepatosplenic, or lymphatic organs. As González-Espinoza et al. (2021) and Preena et al. (2024) have noted, organs such as the lymph nodes, spleen, liver, genital organs, and reticuloendothelial organs are considered "golden organs" for detecting *Brucella* spp. through bacterial isolation (Carvalho et al., 2023). Future approaches will improve the sensitivity of molecular detection by including a wider range of these organs, which may increase the chances of identifying latent or persistent *Brucella* infections in wild long-tailed macaques.

Despite the low prevalence of brucellosis in Malaysian livestock such as goats (1%) and the absence of evidence suggesting livestock-to-wildlife transmission, the detection of a single RBPT-positive result, even if a false positive, highlights the importance of continued surveillance. Zulhisam et al. (2026) similarly argued that while the lack of a human-livestock-wildlife interface may break transmission cycles, routine screening of wildlife brucellosis remains crucial, as it may affect the reproductive performance of wild fauna and have implications for conservation. This risk is further reduced by the Malaysian Department of Veterinary Services‘ proactive vaccination and eradication campaigns against brucellosis in farm animals (Department of Veterinary Services, 2020). Nevertheless, the potential of wild macaques acting as reservoirs for *Brucella* spp. cannot be entirely dismissed, as the bacterium can still cause reproductive issues in both wildlife and livestock, therefore posing a potential risk to both local ecological balance and public health (Godfroid, 2002; Schlabritz-Loutsevitch et al., 2009).

Furthermore, previous studies such as those by Huddleson and Hallman (1929), Mense et al. (2004), and Percy et al. (1972) demonstrated that non-human primates, including macaques, are susceptible to various *Brucella* species such as *B. melitensis*, *B. suis*, *B. abortus*, and *B. canis*. Understanding the exact *Brucella* biovars present in wildlife is

essential, particularly given the potential impact on human health. The findings of this study contribute to the comprehensive understanding of *Brucella* spp. infections in wild long-tailed macaques and underscore the importance of using a variety of diagnostic methods, including serological and molecular tests, to accurately detect and monitor the disease, a point also emphasised by Zulhisam et al. (2026) in their recommendation that serological screening results should always be supported with confirmatory tests. In order to prevent future outbreaks and mitigate the risks posed by *Brucella* spp. in non-human primates, surveillance of human-wildlife interactions should be continued, and wildlife-specific diagnostic methods should be further developed.

CONCLUSION

This study found no confirmed anti-*Brucella* antibodies after CFT verification and no detectable *Brucella* DNA in reproductive tissues, indicating no active infection in the sampled macaques. The combined prevalence of exposure or infection was effectively zero, with the 95% confidence interval upper bound below two per cent. Wild long-tailed macaques in the studied areas of Selangor thus pose a negligible risk for brucellosis transmission to humans. The observed pathological lesions in reproductive and hepatobiliary organs were unrelated to brucellosis. Limitations include convenient sampling and the restriction of molecular analysis to reproductive tissues, leaving lymph nodes, spleen, and liver untested. Future studies should broaden tissue panels, incorporate bacterial culture where safe, and expand zoonotic surveillance to strengthen One Health reinforcement.

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Nutritional Quality of Dominant Herbaceous Forages: A Comparative Study of Burnt and Unburnt Areas of Kikulula Ranch, Tanzania

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ABSTRACT

Herbaceous plants are the primary forage of livestock on rangelands and, hence, are important for the sustainability of ranches. Herbaceous plants are susceptible to fires that may affect forage quality either positively or negatively. This study aimed to evaluate the forage quality of the dominant herbaceous plants in burnt and unburnt areas of Kikulula Ranch (located between 1.5° and 1.8° S and 31.2° and 31.5° E), focusing on crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF). Importance value index of common herbaceous plants and nutrient quality of the three most abundant forage species were computed by standard methods. *Themeda triandra*, *Loudetia kagerensis* and *Desmodium uncinatum* were the most common fodder species. CP levels in *Themeda triandra* and *Desmodium uncinatum* were significantly higher ($p < 0.05$) in burnt areas than in unburnt areas, indicating that burning promotes protein synthesis and growth. Alternatively, in *Themeda triandra*, NDF levels were higher in unburnt areas ($p < 0.05$), indicating reduced forage consumption and digestibility for livestock. On the other hand, ADF levels did not differ significantly between burnt and unburnt areas ($p > 0.05$). In *Desmodium uncinatum*, NDF and ADF contents were not different between the burnt and unburnt areas ($p > 0.05$). Furthermore, in *Loudetia kagerensis,* the levels of CP, NDF and ADF did not differ between burnt and unburnt parts ($p > 0.05$). Hence, the study recommends *Themeda triandra* and *Desmodium uncinatum* as quality forages with high crude protein, particularly in rangelands susceptible to frequent fires.

Keywords: Acid detergent fibre, crude protein, fires, herbaceous plants, neutral detergent fibre

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INTRODUCTION

In Tanzania, the livestock sector contributes to the country's agricultural gross domestic product (GDP) by 7.4% (World Bank, 2024). The real undisclosed cattle operation depends on good ranch management. Animal feeding is an important component of ranch management, which is principally constituted by herbaceous plants (grasses, herbs and forbs). Cattle fodder value is associated with parameters such as nutritional content, biomass output, palatability, growth habit (annual or perennial) and tolerance to grazing pressure (Foran et al. 1978). However, fodder plants' quantity and quality are affected by environmental and management factors such as fire, soil degradation, antinutritional components, mycotoxins and technological constraints (Akintan, 2025; Baris, 2023; Ndah et al., 2017; Thornton et al., 2014; Thapa et al., 2022). Fire has received much attention for its effects on feed availability among these factors. It has been associated with the reduction of quantity and quality of animal feed, affects nutrient cycling and interferes with ecosystem functioning (Reid et al., 2023; Thapa et al., 2022). However, other studies suggest that fire leads to increased nutrient storage and production of higher quality feed (Debyle, 1989; Ranglack et al., 2015; Vera-Velez et al., 2023). Additionally, controlled burning is often used in the management of rangelands and ranches to promote new pastures, eliminate unwanted plants, and manage pests and diseases (Eloy et al., 2019; Israr et al., 2024; Pandit et al., 2021). Little is known of the effect of fire on the nutritional value of the food, and results are variable; therefore, further work is required.

Many plants are adapted to fire and tend to resprout from the base after even severe burns. Pyrophytes or fire-tolerant plants survive with subterranean storage organs or regenerate quickly after fire or possess seeds that require heat or smoke for germination (Gold et al., 2023; Hoffmann et al., 2003; Stephan et al., 2010). Normally, species such as *Panicum maximum*, *Hyparrhenia hirta* and *Chrysopogon plumulosus* are fire-sensitive (Jacobs & Schloeder, 2002; Nepolo & Mapaure, 2012; Ryan & Williams, 2011). Other species like *Themeda triandra*, *Monechma divaricatum*, *Pupalia lappacea* and *Melhania damarana* are fire-adapted and tend to thrive in abundance after fire (Jacobs & Schloeder, 2002; Nepolo & Mapaure, 2012).

Fire stimulates biological processes and growth of plants and thus may variably affect the nutrient levels in plant tissues such as crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Thapa et al., 2022). CP, ADF and NDF reflect forage nitrogen content, digestibility and the amount consumed by grazing animals, respectively. In Nepal, the burning of plant tissues in grasses and post-fire regrowth resulted in increased CP. However, NDF and ADF increased in mature grasses, which in turn decreased digestibility (Thapa et al., 2022). But other studies have been inconsistent on nutritional susceptibility to fires. For example, frequent fire was associated with high abundance of herbaceous, low-crude-protein species in North America (Harrison et al., 2021). Additionally, Snobl

et al. (2022) reported that large wildfires increased the amount and quality of feed in the elk habitat in the United States. Species from routinely burnt locations had crude protein levels up to 45.7% (Harrison et al., 2021). However, in Zambia, Namukonde et al. (2023) reported no significant differences in crude protein, neutral detergent fibre and acid detergent fibre between burnt and unburnt grass species. The variable outcome here reflects the unpredictability and situation-dependent effect of fire on the nutritional value of feed. Fire effects can vary with plant type and environment. The effect of fire on the quality of feed under different climatic conditions needs to be studied.

The Kikulula Ranch in the Kagera Region has reported fires and soil degradation (Nzunda et al., 2013). However, limited investigative studies have been conducted to ascertain the quality of herbaceous plants in the degraded region. This study is therefore on the right track in light of burning occurring in areas of the ranch.

The study was undertaken to: (i) identify the dominant species of herbaceous fodder and (ii) determine the nutritional quality of the dominant species of the burnt and unburnt areas of the ranch. The results will add to the existing knowledge on the effects of fire on the nutritional value of fodder and provide useful information for the development of proper ranch management techniques for the sustainability of animal production.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted in Kikulula Ranch, Karagwe District in Kagera region in north-western Tanzania. It is one of six ranches established in this area for raising livestock. Kikulula spans an area of 12,700 ha and is situated between 1.5° and 1.8° S latitude and 31.2° and 31.5° E longitude (Figure 1). Kikulula is a bimodal rainfall sub-humid grassland with an annual average rainfall of between 750 and 1,250 millimetres (Tanzania Meteorological Agency [TMA], 2023). Rain falls from October to January, and from March to June. Average annual temperature is about 21 ºC, and temperature varies from 15 ºC to 28 ºC (The United Republic of Tanzania, 2019).

The soil is mainly sandstone in the steep hills and clay loam in the flat areas (Merumba et al., 2020). The Kikulula grazing land comprises a great variety of vegetation types: trees, bushes, shrubs, herbs and grasses. The Kikulula Ranch was selected because it has been less scientifically studied than the other ranches in the country. Furthermore, the nutritional value of forage plants in fire-prone environments is complex and unpredictable and not suitable for comparison. Kikulula Ranch has two fire seasons, a long season from June to September and a short season from January to February (unpublished data 2023). Grasslands burn every year either from controlled fires or wildfires, but bushlands and isolated hills rarely burn.

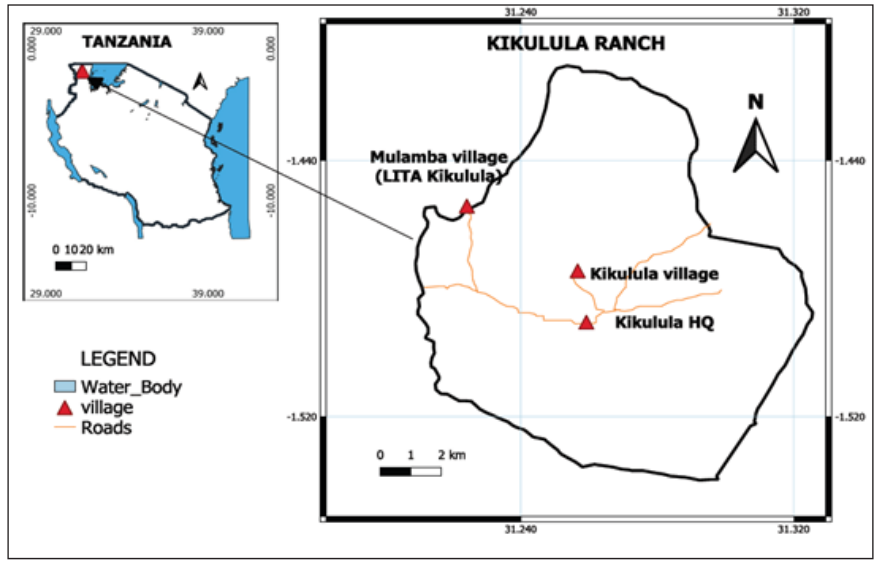


Figure 1. Map Showing Location of Kikulula Ranch in Kagera Region, Tanzania
Source: Field Data using QGIS software Prizren version 3.34 (QGIS Project, 2023)

Study Design

The design used in the study was a two-group simple randomised design under a completely randomised design (Kothari, 2004). The areas were chosen deliberately and were categorised into burnt and unburnt areas. Dominance of the herbaceous species was determined by laying down seven transect lines of 300 metres each and 50 metres apart in each site of hills, plains and bush lowland, giving a total of 21 transects. The quadrat and counting procedures were utilised where a 1 m^2 Daubenmire cover frame was randomly thrown every 10 metres along each transect line according to Caratti (2006). In each quadrat, herbaceous plants were identified carefully to the species level with emphasis on important morphological characters. The herbaceous species were listed in a notebook, with their frequencies of recurrence. Correspondingly, the percentage cover of each item was approximated to calculate the percentage dominance of the plants.

Data Collection

Herbaceous plants were recorded for abundance at species level. The data were collected during the rainy season before the blooming of the herbaceous plants when the nutrient levels are generally high (Kilcher, 1981; Sangu & Nahonyo, 2021). Proximate analysis of the three most abundant forage plant species was done by collecting thirty samples per species, fifteen in the burnt area and the other fifteen in the unburnt area, to give a total of ninety samples for the three species. We targeted the upper leaves most likely to be consumed by animals. Fresh top leaves and grass stems were randomly collected

from the herbaceous plants along a 300-metre transect line according to the methodology recommended by Geisseler et al. (2017). About 250 g was collected for each of fresh top leaves and grass stems.

Data Processing and Analysis

Importance Value Index of Herbaceous Plant Species in Kikulula Ranch+

The importance value index (IVI) for herbaceous species was calculated as per Phillips (1959) as given in Equation 1:

$$\text{IVI} = \text{Relative Density} + \text{Relative Frequency} + \text{Relative Dominance} \quad [1]$$

Crude Protein, Neutral Detergent Fibre and Acid Detergent Fibre in Herbaceous Forage Species in Burnt and Unburnt Areas

The collected samples of leaves and stems of dominant herbaceous forages were packed in paper bags and sent to the Sokoine University of Agriculture laboratory for analysis. The forage samples were air-dried for 24 hours to reduce moisture and prevent loss of nutrients, then oven-dried and chemically analysed in duplicate.

Crude Protein Content

The forage was analysed for CP content by the micro Kjeldahl method. This involved digestion of 1 g ground forage sub-sample in sulfuric acid (H_2SO_4). The samples were oven-dried at 55 °C for 8 hours and analysed for CP according to the 21st edition of the international protocols formulated by the Association of Official Analytical Chemists (Latimer, 2023). Nitrogen was determined in duplicate by distillation and titration of ammonia (NH_3) with a standard solution in the distillation unit model K-300 (Silva et al., 2016). Crude protein was then calculated from the nitrogen level obtained from the ammonia according to Equations 2 and 3.

$$\text{Kjeldahl nitrogen (\%)} = \frac{(V_A - V_B) \times M \times 14.01}{W \times 10} \quad [2]$$

where:

V_A = Volume (mL) of standardised acid (for sample)

V_B = Volume of standardised acid (for blank reagent)

M = Molarity of standard hydrochloric acid (HCl)

14.01 = Atomic weight of nitrogen

W = Weight of test portion

10 = Factor for conversion mg/g to percentage

$$\text{CP} = \text{Total nitrogen (\%)} \times 6.25 \quad [3]$$

Where:
6.25 is a factor for usable protein

Neutral Detergent Fibre

The NDF was analysed by the Automated Fibre Analysis with the Ankom 200 Fibre Analyser according to the method of Van Soest et al. (1991). Dried and ground samples of forage (1 g) were placed in a crucible at room temperature, and 100 mL of neutral detergent solution, 0.5 g of sodium sulfate (Na_2SO_4) and a few drops of octanol ($C_8H_{18}O$) were added. The mixture was then heated to the boil and allowed to reflux for 60 minutes. Then the mixture was filtered and washed three times with water and acetone. Samples were dried at 105 °C for 8 hours, cooled in desiccators and weighed. Results were calculated with Equation 4. Samples were dried in an oven at 55 °C for 8 hours and thereafter analysed for neutral detergent fibre (Latimer, 2023).

$$\text{NDF (\%)} = \frac{W_2 - W_1}{W_3} \times 100 \quad [4]$$

Where:
W_1 = Weight of the empty crucible (g)
W_2 = Weight of crucible + dried NDF residues (g)
W_3 = Weight of oven-dry weight of the sample (g)

Acid Detergent Fibre

The acid detergent fibre content was measured by Ankom 200 Fibre Analyser. Duplicate sub-samples (1 g air-dried ground forage) were added to a beaker containing 100 mL of an acid-detergent solution with a few drops of $C_8H_{18}O$ (Troyer et al., 2020). The mixtures were boiled and refluxed for 60 minutes, filtered and washed with boiling water and acetone. Then the samples were dried at 105 °C for 8 hours and cooled. The weight of the dried sample was calculated from Equation 5.

$$\text{ADF (\%)} = \frac{W_3 - W_1}{W_2} \times 100 \quad [5]$$

where:
W_1 = Weight of the empty crucible (g)
W_2 = Initial dry weight of the test sample (g)
W_3 = Final dry weight of the crucible with the fibre residue after the acid detergent extraction process (digestion and drying) (g)

Burnt and Unburnt Parts of Kikulula Ranch

The status of fire in Kikulula Ranch was derived from prescribed fires and wildfires and from physical characteristics of the landscape. Fire hotspots were extracted from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor with a spatial resolution of 1 kilometre (Giglio et al., 2018).

Statistical Analysis

Statistical analysis was performed by descriptive analysis and equality of means using R version 4.4.2.

RESULTS AND DISCUSSION

Burnt and Unburnt Parts of Kikulula Ranch

Figure 2 shows fire hotspots, which occurred in large plains and hills with no lowland barriers to impede fire spread. These regions are hilly, and the lowlands or depressions between

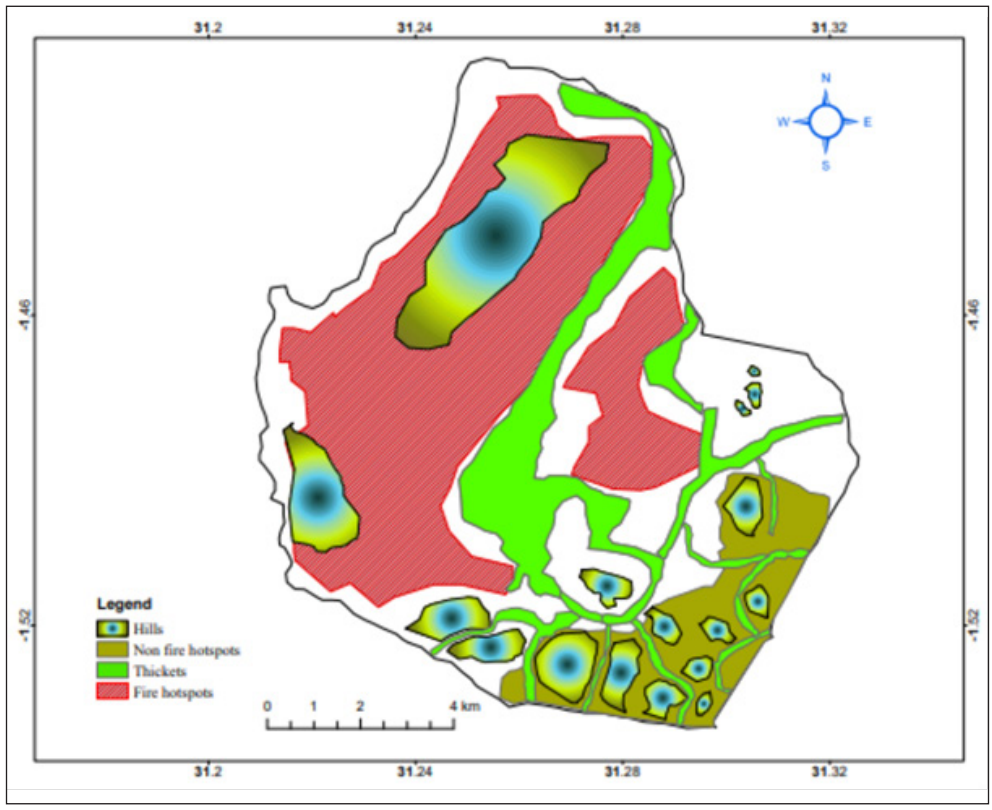


Figure 2. Fire hotspots and non-fire hotspots in Kikulula Ranch (Created by Researchers of this Study)

hills are overgrown with thickets and bushes, which serve as a natural barrier for fire spread over the landscape. By contrast, the non-fire hotspots were plains and hills embedded in a fragmented landscape where lowlands with thickets served as efficient fire barriers.

Composition of Herbaceous Species in Kikulula Ranch

Table 1 shows importance value indices of herbaceous plants in Kikulula Ranch. The results indicated that the three most important forage plants were grasses, *Themeda triandra*, *Loudetia kagerensis* and a leguminous herb, *Desmodium uncinatum*. Results revealed that the herbaceous plant species with an IVI of 56.23 are widely distributed in Kikulula Ranch and well adapted to the prevailing environmental conditions. This is consistent with the results of Sarwatt and Mollel (2006) and Bloesch (2008), who reported *Themeda triandra*, *Setaria sphacelata*, *Cenchrus ciliaris*, *Panicum* spp., *Pennisetum purpureum*, *Loudetia* spp., *Centrosema pubescens*, *Neonotonia wightii* and *Desmodium intortum* in other highlands of the Kagera region.

Table 1
Importance Value Index of herbaceous species in Kikulula Ranch

Herbaceous Species	IVI Percentage
Themeda triandra	16.01
Loudetia kagerensis	7.29
Urochloa mosambiensis	6.29
Cyanodon dactylon	3.85
Abutilon angulatum	3.57
Panicum maximum	3.38
Brachiaria brizantha	3.3
Hyparrhenia rufa	2.48
Desmodium uncinatum	2.44
Sporobolus pyramidalis	2.17
Sporobolus africanus	1.96
Setaria pumila	1.8
Eragrostis tenuifolia	1.7
IVI % of ten herbaceous species	56.23
IVI% of other 115 herbaceous species	35.58

Note. Importance Value Indices were calculated for all recorded herbaceous species, along with the ten most abundant species, at Kikulula Ranch

T. triandra and *L. kagerensis* were ecologically important with their dominance and spreading in different conditions in burnt and unburnt areas with IVI of 23.01 %. The species appear to be well adapted to Kikulula Ranch, perhaps due to a dense network of rhizomes and rapid re-colonisation of burnt areas (Hagl et al., 2021; Hodgson et al., 2024). They have long light awns that aid in dispersal by wind and escape from fire by smoke (Ghebrehiwot et al., 2012; Zahed & Bączek-Kwinta, 2023). Thus, *L. kagerensis* is well suited to the fire-prone hilly habitats of Kikulula Ranch. *L. kagerensis* is a species of montane, fire-resistant, drought-resistant, and grazing-resistant (National Park Service, 2021). *L. kagerensis* is fire-resistant; it bounces back quickly from fire and thrives in fire-prone ecosystems. The spikelets are lightweight and lanceolate and are easily dispersed by wind and smoke, which, due to the open panicle and flexible branches, promote germination and rapid regrowth (Ripley et al., 2015). Its leaves are narrow and slender in blade, and glaucous and pubescent

on the surface to reduce water loss, thus giving it a better chance to survive in nutrient-poor soils. Several studies have reported the dominance of *L. kagerensis* in poor soils of the hilly fire-prone ecosystems of tropical Africa (Kyagaba, 1997).

The high IVI of *D. uncinatum* in the leguminous herbs could be associated with its invasive nature and fast growth rate, which help it outcompete the native vegetation (Witt & Luke, 2017). It has allelopathic properties, with high nitrogen-fixing ability and tolerance to a range of environmental stresses (Hooper et al., 2015; Ngondya et al., 2017). *D. uncinatum* is a tropical American species introduced in the 1990s in Kikulula Ranch under the Kagera Livestock Development Programme to maximise milk yield (Komwihangilo et al., 2011). Witt and Luke (2017) studied the effects of the invasive species *D. uncinatum* on plant community structure, biodiversity and ecosystem function in East Africa. However, *D. uncinatum* is a high crude protein species (Heuzé et al., 2015). Thus, it is important to manage the grazing land in a sustainable way, increase the production of herbaceous forages with high nutritional value and control the invasion of this species by mechanical removal, burning and targeted grazing.

Nutrient Quality of *Themeda triandra* Forssk. in the Burnt and Unburnt Areas

Themeda triandra CP and NDF percentage values were significantly different between the burnt and unburnt areas (Table 2; $t = 2.91$, $F = 55.2$, $p < 0.05$; $t = 2.96$, $F = 1.69$, $p < 0.05$, respectively). The higher CP level in *Themeda triandra* in the burnt area shows that the plant is fire-tolerant and has a high rate of amino acid and protein synthesis for its growth. For example, seed culms of *T. triandra* were observed to remain standing and unburnt after hot fires (Snyman, 2013). Our results agree with Reid et al. (2023). Also, the low NDF value of *T. triandra* in the burnt area showed a decrease in digestible cell wall content in the young leaves after fires, which may limit vegetation intake by animals. These data also indicate that fire affects the cellulose and hemicellulose fractions of NDF. The low energy content of young leaves is due to low hemicelluloses, cellulose and lignin, which are components of the NDF (Bin et al., 2021). Fire tolerance in *T. triandra* may also be due to its ability to resprout from scars after burning. The increase in fibre content is due to the dead mass spots of growing forage plants (Erkovan et al., 2009). ADF levels in *T. triandra*, however, did not differ significantly between burnt and unburnt sites ($t = 1.37$, $F = 3.73$, $p > 0.05$). The ADF seems to be a problem with hemicellulose, which is a factor in the digestibility of forage, and is exacerbated by fires. Fire does not influence the lignin content in ADF significantly, and therefore similar values are found in burnt and unburnt areas. Lignin and fibres have a known defensive role and reduce the quality of digestible forage plants (Lee et al., 2017). The fibre contents of *Themeda triandra* are similar to those reported by Lunt and Morgan (1999) and Erkovan et al. (2009). The ADF contents in forage plants from the burnt and unburnt areas are consistent with the observations reported by Pule et al. (2023).

Table 2
Variation of selected forage qualities in Themeda triandra between burnt and unburnt areas

Nutrition Parameter (%)	Levene's Test for Equality of Variances		t-test for Equality of Means		Mean	
	F	Sig	t-test (2-tailed)	Sig.	Burnt	Unburnt
NDF	1.69	0.2	2.96	0.006	68.86 ± 0.39	71.71 ± 0.6
ADF	3.73	0.06	1.37	0.18	36.06 ± 0.49	37.32 ± 0.77
CP	55.2	$4\ e^{-8}$	2.91	0.007	6.22 ± 0.06	5.41 ± 0.27

Note. Values of NDF, ADF and CP in *Themeda triandra*

Nutrient Quality of *Loudetia kagerensis* (K.Schum.) C.E.Hubb. ex Hutch. in Burnt and Unburnt Areas

The percentage levels of NDF, ADF and CP in *Loudetia kagerensis* revealed no significant differences between the burnt and unburnt areas (Table 3; t = 2.96, F = 0.12, $p > 0.05$; t = 1.37, F = 0.36, $p > 0.05$; t = 2.91, F = 0.47, $p > 0.05$, respectively). In both environments, *Loudetia kagerensis* had higher levels of NDF and ADF than CP (though not statistically significant), indicating a higher fibre content of the forage necessary for mechanical support but decreasing vegetation consumption and digestibility. A high ratio of carbohydrate (fibre) to nitrogenous compounds (protein) also indicates maturity of the plant. The results are in agreement with Kyagaba (1997), who recorded low values of nutrients for *L. kagerensis* but good growth in burnt environments. The similarity of nutritive values of *L. kagerensis* in burnt and unburnt areas may be due to inherent characteristics of the species. Heat Shock Proteins (HSPs) genes are reported to be linked to stable nutritional allocation (Mas-ud et al., 2025), as well as microRNAs such as miR156 (Kim et al., 2012). Field et al. (1973) reported low acceptability of *L. kagerensis* by Ankole cattle and buffaloes in southern Uganda. This indicates that *L. kagerensis* is a poor forage for livestock nutrition even in fire-managed ecosystems. However, it is a very hardy species and well adapted to fire-prone ecosystems. It plays important ecological roles such as stabilising soil on sloping hills.

Table 3
Variation of selected forage qualities in Loudetia kagerensis between burnt and unburnt areas

Nutrition Parameter (%)	Levene's Test for Equality of Variances		t-test for Equality of Means		Mean	
	F	Sig.	t-test (2-tailed)	Sig.	Burnt	Unburnt
NDF	0.12	0.73	2.96	0.4	73.57 ± 0.8	74.461 ± 0.66
ADF	0.36	0.55	1.37	0.29	37.5 ± 0.64	38.43 ± 0.58
CP	0.47	0.5	2.91	0.12	6.26 ± 0.25	5.68 ± 0.25

Note. Values of NDF, ADF and CP in *Loudetia kagerensis*

Nutrient Quality of *Desmodium uncinatum* (Jacq.) DC. *in* Burnt and Unburnt Areas

The mean CP of *D. uncinatum* was significantly higher in burnt than unburnt areas (Table 4; t = 2.6, F = 3.02, $p < 0.05$). This result shows that *Desmodium uncinatum* is fire-tolerant and biomass accumulation is due to active protein synthesis in the burnt areas. Likewise, at the genus level, similar results were recorded by Mligo (2019) in *Desmodium barbatum* that regenerated after frequent fires in Pugu Forest Reserve. Also, Carbone and Aguilar (2016) reported that in the burnt area of Argentina, *Desmodium uncinatum* had high total nitrogen. In this case, CP is an essential component of total nitrogen.

The study findings also support the observations of Mathenia (2011), Nichols et al. (2021), Thapa et al. (2022) and Haynes et al. (2023), who reported increased nutritive values of forage species after fire events. Moreover, there was no significant difference in the values of NDF and ADF in *Desmodium uncinatum* between burnt and unburnt areas, as shown in Table 4 (t = 1.11, F = 1.88, $p > 0.05$; t = 0.5, F = 9.29, $p > 0.05$, respectively). Moderate fire conditions are unlikely to have much effect on fibre formation of cellulose, hemicellulose and lignin in *D. uncinatum*. Hacker (1992) reported that *D. uncinatum* recovered from moderate fires, but was severely damaged by intense fires. This resilience can be attributed to the ability of fire to stimulate new growth in young herbaceous plants that are generally less fibrous (Thorvaldsson et al., 2007) but have active enzymes that synthesise amino acids and promote fast growth and development. The results of NDF and ADF of *D. uncinatum* are consistent with the results of Carbone and Aguilar (2016), who found that leaf biomass, which determines the fibre content, was not affected in the burnt areas.

Table 4
Variation of selected forage qualities in the Desmodium uncinatum between burnt and unburnt areas

Nutrition Parameter (%)	Levene's Test for Equality of Variances		t-test for Equality of Means		Mean	
	F	Sig.	t-test (2-tailed)	Sig.	Burnt	Unburnt
NDF	1.88	0.18	1.11	0.28	53.45 ± 0.46	54.31 ± 0.62
ADF	9.29	0.005	0.5	0.62	28.05 ± 0.39	27.52 ± 0.99
CP	3.02	0.093	2.6	0.02	19.25 ± 0.45	17.91 ± 0.25

Note. Values of NDF, ADF and CP in *Desmodium uncinatum*

CONCLUSION

In the study area, the dominant forage grasses were *Themeda triandra* and *Loudetia kagerensis,* while the dominant leguminous species was *Desmodium uncinatum*. These taxa are able to survive in burnt and unburnt habitats and highlight their resistance to numerous

ecological stressors of ranching activities such as grazing, controlled burns, and wildfires. *Themeda triandra* and *Desmodium uncinatum* in burnt areas provided substantial crude protein. *Themeda triandra* in the burnt areas had low neutral detergent fibre, confirming high protein content but poor digestible cell wall energy. Levels of crude protein, neutral detergent fibre and acid detergent fibre of *Loudetia kagerensis* were, however, not different between burnt and unburnt areas. *Desmodium uncinatum* similarly revealed higher crude protein levels in burnt areas, but the neutral detergent fibre and acid detergent fibre levels were similar in both parts.

Controlled or prescribed burning is one option that can be employed on ranches to improve the quality of pastures (especially protein content in *Desmodium uncinatum* and *Themeda triandra*). However, fire reduces the neutral detergent fibre content of *Themeda triandra*, a measure of digestible cell wall components (cellulose, hemicellulose and lignin), and so reduces the energy-yielding capability of the forage. Fire did not affect levels of crude protein, acid detergent fibre and neutral detergent fibre in *Loudetia kagerensis* and neutral detergent fibre and acid detergent fibre levels in *Desmodium uncinatum*. Composition, palatability and quality of herbaceous species are key factors for the amount of vegetation consumed, herbivorous biomass and reproductive success (Herrero et al., 2015; Wold et al., 2023).

IMPLICATION OF THE STUDY

Based on the findings, this study has the following implications for the management of ranches and ecological sustainability: Fire is a useful tool in improving rangeland forages, especially *Themeda triandra* and *Desmodium uncinatum,* by increasing levels of crude protein. Strategic burning increases forage plant nutritional values. Burning increases protein content and decreases neutral detergent fibre content in *Themeda triandra*, so selective management of forage is needed. This is a trade-off with forage higher in protein but with less digestible cell wall energy. Fire responses are species-specific. Not all species respond the same to fire. Fire increases the nutritive value of *Themeda triandra* and *Desmodium uncinatum*. Conversely, the nutrient composition (crude protein, acid detergent fibre, neutral detergent fibre) of *Loudetia kagerensis* was similar between burnt and unburnt areas, suggesting that fire does not provide the same nutritional benefits to this grass species. The dominant species are very resilient, particularly *Themeda triandra*, *Loudetia kagerensis* and *Desmodium uncinatum* that can thrive in burnt and unburnt areas, despite grazing and fire. Dependable, consistent forage options for ranchers. Finally, the paper comments on better performance of livestock. The increased protein content of *Desmodium uncinatum* and *Themeda triandra* in burnt areas is likely to improve the health of livestock and thus increase biomass and reproductive success of herbivores grazing in burnt areas.

LIMITATION AND RECOMMENDATION FOR FUTURE RESEARCH

The nutritional composition of the herbaceous forage plants and other plant forms can be altered by edaphic variables, light, space and water (Lascano et al., 2001; Melo et al., 2022), but these aspects were not evaluated in the present study. Where high-protein, low-fibre forages are required, it is recommended that strategic burning is used to increase protein availability if the energy-yielding fibre (NDF) content of the overall diet is not reduced to a great extent.

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Development and Evaluation of Fermented Sago-Based Poultry Feed Using Solid-State Fermentation with *Aspergillus niger*

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ABSTRACT

Sago pith is a locally available, energy-rich feed; however, its protein content is low, and thus it cannot be used in broiler feeds. This study aimed to assess the use of solid-state fermentation (SSF) using *Aspergillus niger* as an approach to enhance sago for poultry feed. Sago was fermented using *A. niger,* and diets of 10%, 20%, and 30% fermented sago and a commercial control were tested in a 42-day trial in comparison to a lower protein negative control. One hundred Cobb 500 chicks were randomly assigned to the five treatments, and growth, along with feed conversion and carcass characteristics, were recorded after 42 days. The crude protein increased to 12.4% while the crude fibre decreased to 5.2% after fermentation. There was no statistically significant difference was detected between the 20% diet and the commercial control in final body weight (2.42 kg and 2.50 kg, respectively), or in FCR (1.93 and 1.97, respectively). The 10% and 20% groups were about 73-74% dressed, whereas the lower-protein control was 72.3%. The nutrient balance was limiting at 30% inclusion due to nutrient limitation. Nutrient quality of sago was enhanced by the *A. niger* fermentation, and broiler performance was not significantly affected by the inclusion of fermented sago up to 20%. Economic benefits depend on the prices of ingredients and the production, sterilisation, drying, labour and quality control costs of inoculum.

Keywords: Aspergillus niger, alternative feedstuffs, broiler chickens, carcass yield, growth performance, sago pith, solid-state fermentation

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INTRODUCTION

High feed prices and reliance on imported feedstuffs such as maize and soybean meal are ongoing challenges for poultry production in tropical regions. The use of locally available alternative feed resources may help reduce dependence on conventional ingredients and improve the sustainability of poultry production (Babatunde et al., 2021). The sago palm (*Metroxylon sago*) is widely grown in Southeast Asia, with its starchy pith, known as sago, being a potential local energy source for animal feeds. Sago is a very rich source of carbohydrates, but is very unbalanced in terms of nutritional composition. Ground sago pith has low crude-protein content and relatively high fibre, which limits its nutritional value as a poultry-feed ingredient (Ab Jalil et al., 2015). These nutritional limitations indicate that appropriate processing or supplementation is required before sago can be effectively incorporated into nutritionally balanced poultry diets.

One possible way to improve the nutritive value of low-quality feedstuffs like sago starch is the use of filamentous fungus in solid-state fermentation (SSF). SSF consists of growing microorganisms in moist solid media, which leads to growth and changes in the substrate due to enzyme activity. Previous studies have demonstrated the potential of fermentation to improve sago-derived feed materials. Ab Jalil et al. (2015) reported an increase in crude protein and a reduction in crude fibre following SSF of ground sago pith with *Rhizopus oligosporus*. Similarly, Lani et al. (2021) reported that mixed-culture fermentation of sago waste increased crude protein by approximately 1.3 percentage points and reduced crude fibre, with the fermented product subsequently used as a partial feed ingredient for red hybrid tilapia. These findings suggest that microbial fermentation can improve the nutritional characteristics of sago-based substrates.

Filamentous fungi such as *Aspergillus niger* are particularly suitable for SSF because they produce extracellular enzymes capable of degrading complex components of plant materials. During SSF, *A. niger* can produce enzymes including cellulases, xylanases, proteases and phytases, which may contribute to changes in substrate composition and nutrient availability (Shi et al., 2015). Fermentation may also generate organic acids and other metabolites that alter substrate pH. More broadly, studies of fermented poultry feeds have reported reductions in antinutritional factors and changes in gut microbial communities, with potential benefits for growth and health (Predescu et al., 2024; Zhu et al., 2023). These properties indicate that fermentation has potential to improve sago pith beyond its value as a carbohydrate source.

The present study aimed to develop a fermented sago-based poultry feed using *Aspergillus niger* for solid-state fermentation and to evaluate the performance of broilers and carcass characteristics when fed with this fermented poultry feed. We hypothesised that the fermentation of sago pith using *A. niger* would greatly improve the crude protein and nutritional value of the sago pith and that it could be incorporated into the diets at

a moderate level (10-20%) without negative effects on growth performance and meat production. The feeding study was designed to evaluate the effect of fermented sago at different inclusion levels (0%, 10%, 20%, 30%) on broiler development, feed efficiency and carcass characteristics in comparison with a control (regular commercial diet) and a negative control (0% inclusion diet). This study combines the enrichment of a starch-based substrate with *A. niger* with the addition of graded amounts of fermented sago to broiler diets and the measurement of growth efficiency and carcass yield. This integrated design recognises a practical inclusion level and meets the requirement for locally available feed ingredients in the sago-producing areas.

MATERIALS AND METHODS

Sago Substrate and Inoculum Preparation

Sago pith was collected from the East Campus plantation of Universiti Malaysia Sarawak (UNIMAS), dried and milled. Distilled water was added progressively to achieve 50% moisture (w/w), after which the substrate was divided into 5-kg batches and sterilised at 121 °C for 20 min. *Aspergillus niger* PAN 1, supplied by the Molecular Genetic Laboratory, UNIMAS, was cultured on potato dextrose agar at 30 °C for 5-7 days. Conidia were harvested in sterile distilled water containing 0.1% Tween 80 and counted using a haemocytometer. Each 5-kg batch received 250 mL of spore suspension at 2×10^8 spores/mL.

Solid-State Fermentation

The inoculated substrate was spread in layers approximately 2-3 cm thick and incubated aerobically at 30 °C for five days. The material was mixed and aerated daily, while temperature and visible contamination were monitored. Fermentation was terminated by oven-drying at 60°C for 24-36 h. The dried product was milled and stored in sealed containers until analysis and feed preparation.

Experimental Diets and Feed Preparation

Five dietary treatments were evaluated: a commercial positive control (PC), a negative control (NC) without fermented sago, and diets containing 10%, 20% or 30% fermented sago (FS). The PC provided approximately 21% crude protein (CP) and 3100 kcal/kg metabolizable energy (ME), consistent with recommended broiler nutrient levels (National Research Council [NRC], 1994). The NC contained approximately 18% CP and 3000 kcal/kg ME and served as the lower-protein reference diet.

In the formulated diets, fermented sago progressively replaced yellow maize, while the fish meal/protein source, rice bran, vitamin-mineral premix and vegetable or palm

oil were maintained at constant proportions. Previous studies have shown that solid-state fermentation can improve the nutritive value of sago pith for poultry feed and that fermented alternative feed ingredients can partially replace maize in broiler diets (Ab Jalil et al., 2015; Aladi et al., 2021). The diets were formulated using least-cost principles and standard amino-acid balancing practices (Moran et al., 1992).

All ingredients were mixed thoroughly and processed into a uniform physical form. Figure 1 shows the pelletizer used during feed preparation. The use of locally available alternative ingredients was also considered relevant to reducing reliance on conventional feed ingredients (Babatunde et al., 2021).



Figure 1. Pelletizer machine producing uniform feed pellets

Chemical and Nutritional Analyses

Representative samples of unfermented sago, fermented sago and each experimental diet were analysed in triplicate. Dry matter, crude protein, crude fibre, ether extract and ash were determined according to Latimer (2019). Crude protein, crude fibre, ether extract and ash followed AOAC methods 990.03, 978.10, 920.39 and 942.05, respectively. Ash was determined by incineration at 550°C for 4 h. Nitrogen-free extract (NFE) was calculated using Equation 1.

$$\text{NFE (\% DM)} = 100 - [\text{crude protein} + \text{crude fibre} + \text{ether extract} + \text{ash}] \quad [1]$$

For pH determination, 10 g of sample was suspended in 50 mL of distilled water and measured using a calibrated digital pH meter. ME was estimated from ingredient composition using poultry nutrient values reported by National Research Council (NRC) (1994). The analysed nutrient composition of the experimental diets is presented in Table 1.

Table 1
Proximate composition of experimental broiler diets (mean ± SD)

Diet Treatment	Crude Protein (%)	Crude Fibre (%)	Ether Extract (%)	Ash (%)	Moisture (%)	Calculated ME (kcal/kg)
Positive Control (Commercial)	21.0 ± 0.1	5.0 ± 0.1	8.0 ± 0.2	8.0 ± 0.1	13.0 ± 0.1	~3100
Negative Control (0% Sago)	18.2 ± 0.2	4.3 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	11.3 ± 0.2	~3000
10% Fermented Sago Diet	19.0 ± 0.3	3.9 ± 0.2	6.2 ± 0.1	6.2 ± 0.2	10.5 ± 0.3	~3010
20% Fermented Sago Diet	19.4 ± 0.2	4.4 ± 0.2	6.3 ± 0.1	6.4 ± 0.1	10.6 ± 0.1	~3020
30% Fermented Sago Diet	19.4 ± 0.1	4.7 ± 0.1	6.4 ± 0.2	6.4 ± 0.1	10.6 ± 0.2	~3030

Note. FS = fermented sago; ME values were estimated from ingredient composition

Birds, Ethics and Experimental Design

One-hundred-day-old, unsexed Cobb 500 broiler chicks with an average initial body weight of approximately 42 g were individually tagged and allocated by weight-balanced randomisation to five treatments, with 20 birds per treatment. Birds were housed individually in partitioned wire-floored cages providing approximately 0.5 m² per bird. The protocol was approved by the UNIMAS Animal Ethics Committee (approval no. UNIMAS/AEC/R/F07/079), and all husbandry and slaughter procedures followed the approved animal-welfare requirements. Representative images of the chickens on Days 1, 4, and 11 are shown in Figure 2.

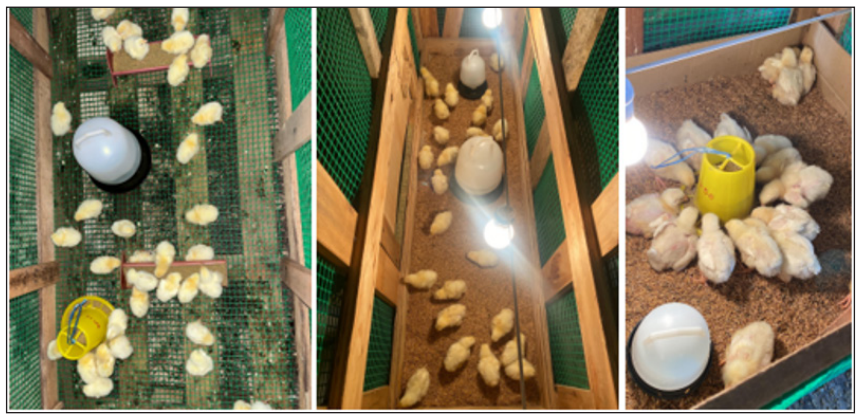


Figure 2. a) Day 1 chicks, b) Day 4 chicks, c) Day 11 chicks

Bird Management and Growth-Performance Measurements

Brooding temperature was maintained at 32 °C for the first 14 days, followed by ambient tropical temperatures of 25-32 °C with fan-assisted ventilation. Lighting was provided for 23 h/day. Birds received a commercial starter diet containing approximately 22% CP during the first seven days. Experimental diets were introduced from day 8 and fully provided from day 10 to day 42. Feed and water were offered *ad libitum*. Vaccinations against Newcastle disease and infectious bursal disease were administered on days 5 and 10, respectively, and no antibiotics or growth promoters were used.



Figure 3. Digital weighing scale

Individual body weight and feed intake were recorded weekly using a digital scale. Health, feeding behaviour and mortality were monitored daily. Body-weight gain, average daily gain (ADG) and feed conversion ratio (FCR) were calculated using Equation 2 and Equation 3. The digital weighing scale used for body-weight measurement is shown in Figure 3.

Body-weight gain = final body weight - initial body weight

ADG (g/bird/day) = body-weight gain (g) / 42 days [2]

FCR = total feed intake (g) / total body-weight gain (g) [3]

For mortality-adjusted FCR, partial intake and body-weight data from birds that died were excluded from the final endpoint calculation (Ramli et al., 2024).

Carcass Evaluation

On day 42, eight birds per treatment were fasted for 6 h and humanely slaughtered by neck incision and exsanguination. Live weight at slaughter, carcass weight, breast weight, thigh weight, drumstick weight and abdominal fat were recorded using standard procedures (Ghazi et al., 2024). Dressing percentage was calculated using Equation 4.

$$\text{Dressing percentage (\%)} = [\text{carcass weight} / \text{live weight at slaughter}] \times 100 \quad [4]$$

Statistical Analysis

Data are presented as mean ± standard deviation. The individual bird was the experimental unit for growth-performance data, and the individual carcass was the experimental unit for carcass traits. Final performance and carcass data were analysed using one-way analysis of variance, with dietary treatment as a fixed effect. Type III sums of squares were applied when unequal sample sizes resulted from mortality. Weekly body weight and feed intake were evaluated using one-way analysis of variance. When treatment effects were significant, means were compared using Tukey's honestly significant difference test. Normality and homogeneity of variance were assessed before analysis. Percentage data were arcsine square-root transformed when required, although untransformed means are reported. Analyses were performed using SPSS version 25 at $p < 0.05$.

RESULTS

Nutrient Enhancement through Fermentation

After five days of solid-state fermentation with *Aspergillus niger*, the crude protein content of sago pith increased from 3.8% to 12.4% on a dry-matter basis. Crude fibre decreased from 6.0% to 5.2%, whereas ether extract increased slightly from 0.5% to 0.7%. Ash increased from 2.3% to 5.1%, nitrogen-free extract decreased from approximately 87.4% to 76.6%, and pH decreased from 6.6 to 5.2. The dry-matter content of the fermented product after drying was 90.5%. The proximate composition of sago pith before and after fermentation is summarised in Table 2.

Table 2
Proximate composition of sago pith before and after A. niger fermentation (dry matter basis)

Component	Unfermented Sago	Fermented Sago (5 days)
Dry Matter (% as fed)	85.0%	90.5% (after drying)
Crude Protein (% DM)	3.8%	12.4%
Crude Fibre (% DM)	6.0%	5.2%
Ether Extract (% DM)	0.5%	0.7%
Ash (% DM)	2.3%	5.1%
Nitrogen-Free Extract (% DM)	~87.4%	~76.6%
pH (20% (w/v) suspension)	6.6 (neutral)	5.2 (slightly acidic)

Note. Values for unfermented sago represent the raw dried sago pith substrate; fermented sago values are after 5 days SSF and drying

Diet Composition and Nutrient Intake

The analysed composition of the experimental diets is presented in Table 1 in the Materials and Methods section. The commercial positive-control diet contained 21.0% crude protein and approximately 3100 kcal/kg metabolizable energy, whereas the negative-control diet contained 18.2% crude protein and approximately 3000 kcal/kg. The diets containing 10%, 20% and 30% fermented sago contained 19.0%, 19.4% and 19.4% crude protein, respectively, with calculated metabolizable-energy values of approximately 3010-3030 kcal/kg. Across the fermented-sago diets, crude fibre ranged from 3.9% to 4.7%, ether extract from 6.2% to 6.4%, ash from 6.2% to 6.4%, and moisture from 10.5% to 10.6%.

Growth Performance

Initial body weights were similar among treatments, ranging from 0.0415 to 0.0423 kg. At day 42, the positive-control group had the highest final body weight (2.500 +/- 0.050 kg), followed by the 20% fermented-sago group (2.421 +/- 0.054 kg) and the 10% fermented-sago group (2.310 +/- 0.052 kg). The negative-control group had the lowest final body weight (1.902 +/- 0.035 kg; $p < 0.001$), while no significant difference was detected between the 20% fermented-sago and positive-control groups ($p > 0.1$). Body-weight gain followed the same pattern, and the 20% fermented-sago group did not differ significantly from the positive control ($p = 0.15$).

Total feed intake was highest in the positive-control group (4.85 kg) and lowest in the negative-control group (4.10 kg). The 20% fermented-sago group consumed more feed than the negative-control group ($p < 0.01$). Feed conversion ratio was lowest in the 20% fermented-sago group (1.93), followed by the 10% fermented-sago (1.96) and positive-control (1.97) groups. The 30% fermented-sago and negative-control groups recorded FCR values of 2.13 and 2.20, respectively. Significant FCR differences were detected between the negative control and the 10% and 20% fermented-sago groups ($p < 0.05$). Two deaths were recorded in the negative-control group, whereas no mortality occurred in the positive-control or fermented-sago groups. No clinical signs of mycotoxicosis were observed during the trial. The growth-performance results for all treatment groups are summarised in Table 3.

Carcass Traits and Meat Yield

The positive-control group had the highest carcass weight (1940 +/- 45 g), whereas the negative-control group had the lowest (1376 +/- 35 g). Carcass weights for the 10%, 20% and 30% fermented-sago groups were 1707 +/- 50 g, 1775 +/- 55 g and 1489 +/- 35 g, respectively. The 10% and 20% fermented-sago groups had higher carcass weights than the negative control ($p < 0.05$). Dressing percentage was highest in the positive-control group (77.6%), compared with 72.3% in the negative control and 73.9%, 73.3% and 73.3% in the 10%, 20% and 30% fermented-sago groups, respectively.

Table 3
Growth performance of broiler chickens fed fermented sago diets vs. control diets (Day 0-42)

Performance Metric	Positive Control (21% CP)	Negative Control (18% CP)	10% Fermented Sago	20% Fermented Sago	30% Fermented Sago
Initial Body Weight (kg)	0.0423 ± 0.0020	0.0421 ± 0.0015	0.0415 ± 0.0017	0.0418 ± 0.0018	0.0417 ± 0.0016
Final Body Weight (kg)	2.500 ± 0.050	1.902 ± 0.035	2.310 ± 0.052	2.421 ± 0.054	2.033 ± 0.030
Body Weight Gain (kg)	2.458 ± 0.050	1.860 ± 0.035	2.269 ± 0.052	2.379 ± 0.054	1.992 ± 0.030
Total Feed Intake (kg)	4.85 ± 0.10	4.10 ± 0.08	4.45 ± 0.09	4.60 ± 0.10	4.25 ± 0.07
Feed Conversion Ratio (FCR)	1.97 ± 0.04	2.20 ± 0.05	1.96 ± 0.05	1.93 ± 0.04	2.13 ± 0.05
Mortality (out of 20 birds)	0	2	0	0	0



Figure 4. Representative broiler carcass cuts collected on day 42, showing breast, thigh, drumstick, and wing portions used for carcass-yield evaluation

Representative broiler carcass cuts collected on day 42 are shown in Figure 4. Breast-muscle weights were 620, 496, 550, 530 and 500 g in the positive control, negative control, 10%, 20% and 30% fermented-sago groups, respectively. Total leg weights were 620, 514.5, 550, 540 and 524.5 g, respectively. Abdominal fat ranged from 1.5% to 1.8% of carcass weight and did not differ among treatments. The carcass characteristics of broilers across all dietary treatments are summarised in Table 4.

Table 4
Carcass characteristics of broilers fed fermented sago diets vs. controls (n = 8 per treatment)

Parameter	Positive Control	Negative Control	10% FS Diet	20% FS Diet	30% FS Diet
Live Weight at Slaughter (g)	2500 ± 50	1902 ± 40	2310 ± 52	2421 ± 54	2033 ± 30
Carcass Weight (g)	1940 ± 45	1376 ± 35	1707 ± 50	1775 ± 55	1489 ± 35
Dressing Percentage (%)	77.6 ± 0.5	72.3 ± 0.6	73.9 ± 0.7	73.3 ± 0.5	73.3 ± 0.7
Breast Muscle Weight (g)	620 ± 10	496 ± 8	550 ± 10	530 ± 9	500 ± 10
Thighs (both) Weight (g)	330 ± 5	274.5 ± 5	290 ± 5	290 ± 5	279.5 ± 5
Drumsticks (both) Weight (g)	290 ± 5	240 ± 5	260 ± 5	250 ± 5	245 ± 5
Total Leg (Thigh+Drum) Weight (g)	620	514.5	550	540	524.5
Abdominal Fat (% of carcass)	1.8 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	1.6 ± 0.2

DISCUSSION

Nutritional Changes Following Fermentation

Solid-state fermentation substantially improved the protein value of sago pith. The increase from 3.8% to 12.4% crude protein represented an increase of 8.6 percentage points, or approximately 3.3 times the original concentration. This improvement is consistent with reports that solid-state fermentation with *Aspergillus niger* can increase crude protein and improve the nutritional quality of plant-based feed substrates (Shi et al., 2015). The increase observed in the present study was also greater than the 1.3% increase in crude protein reported by Lani et al. (2021) following mixed-culture fermentation of sago waste, which may reflect differences in fungal strain, inoculum density, substrate preparation and fermentation conditions. During solid-state fermentation, *A. niger* can produce enzymes such as endoglucanase, xylanase, acid protease and phytase that contribute to the modification of the fermented substrate (Shi et al., 2015).

The reduction in crude fibre is consistent with enzymatic degradation of structural carbohydrates during fermentation. *Aspergillus* species produce a broad range of enzymes capable of degrading plant cell-wall polysaccharides, including cellulolytic and xylanolytic enzymes (de Vries & Visser, 2001). Enzymatic hydrolysis of lignocellulosic components in sago pith residue has also been demonstrated using cellulolytic enzymes (Linggang et al., 2012). In addition, phytase produced by *A. niger* can improve the availability of phytate phosphorus and associated minerals (Gujar et al., 2013). The reduction in pH from 6.6 to 5.2 may reflect the production of organic acids, including citric acid, during *A. niger* fermentation (Lu et al., 1997). Although a lower pH may assist product preservation and could influence gastrointestinal conditions, storage stability and gut-health effects were not measured in this study.

Growth Performance and Nutrient Adequacy

Broilers receiving 10% and 20% fermented sago performed better than birds fed the lower-protein negative-control diet. The 20% fermented-sago diet produced final body weight, body-weight gain and FCR values for which no significant difference was detected relative to the commercial positive control. This finding should be interpreted as comparable performance under the tested conditions rather than proof of nutritional equivalence. The response may have been supported by the increased microbial protein content and enzymatic modification of the fermented substrate, which can improve nutrient availability in fermented feeds (Hombegowda et al., 2021).

Performance declined at 30% inclusion despite a calculated energy value similar to those of the lower-inclusion diets. Higher inclusion may have altered dietary bulk, palatability, nutrient density or amino-acid balance, although these factors were not measured directly. Ralahalu et al. (2021) similarly reported lower body-weight gain in

native chickens receiving diets containing 5-15% fermented sago waste than in birds receiving the control ration, indicating that performance may depend on inclusion level and overall ration formulation. Lani et al. (2021) also demonstrated that fermented sago waste could be used as a partial feed ingredient in tilapia diets, with inclusion of up to 150 g/kg producing growth performance that did not differ significantly from the control.

The commercial starter diet supplied during the first seven days contained approximately 22% crude protein. Thereafter, the 10% and 20% fermented-sago diets contained 19.0-19.4% crude protein and approximately 3010-3020 kcal/kg metabolizable energy. These values were broadly comparable with phase-specific nutritional recommendations for Cobb 500 broilers. For example, Cobb-Vantress (2022) recommends approximately 21-22% crude protein for the starter phase, 19-20% for Grower 1 and 18-19% for Grower 2, with corresponding metabolizable-energy recommendations of approximately 2900, 2950 and 3020 kcal/kg, respectively. The poorer performance of the 30% group therefore suggests that total crude protein and calculated energy alone may not fully describe the nutritional limitations associated with a high fermented-sago inclusion level.

Carcass Responses

Carcass responses followed the pattern observed for growth performance. Birds receiving 10% and 20% fermented sago had greater carcass and muscle weights than the negative-control birds, while the 30% group showed a weaker response. The higher breast and leg weights at moderate inclusion levels may be associated with improved availability of dietary protein and amino acids following fermentation (Hombegowda et al., 2021). However, amino-acid digestibility and muscle-protein deposition were not measured directly. Differences from the commercial control may also reflect the lower crude-protein concentration and possible amino-acid limitations in the fermented-sago diets. Differences from the commercial control may also reflect the lower crude-protein concentration and possible amino-acid limitations of the fermented-sago diets. Reduced-protein broiler diets generally require careful amino-acid balancing to maintain performance and carcass development (Aftab et al., 2006).

Abdominal fat remained below 2% of carcass weight and did not differ among treatments. This indicates that the observed differences in body and carcass weight were not accompanied by detectable changes in abdominal-fat deposition. Improved nutrient use following microbial fermentation has been proposed as one mechanism supporting lean-tissue growth in animals fed fermented ingredients, although this mechanism requires direct confirmation in broilers fed fermented sago.

Economic and Practical Implications

The 20% fermented-sago diet reduced the proportion of conventional maize while maintaining performance that did not differ significantly from the commercial control. This

result suggests potential for reducing dependence on imported feed ingredients in sago-producing regions. Malaysia's corn imports for 2025/2026 are forecast at approximately 3.8 million metric tonnes (Mohd Sait, 2025), while feed commonly accounts for approximately 60-70% of poultry-production costs (Wongnaa et al., 2023). Greater utilisation of locally available sago may therefore contribute to feed-supply resilience while supporting local livelihoods and rural development in sago-producing regions (Konuma, 2018).

Because contemporary ingredient and processing prices were not available, economic performance was evaluated using a break-even relationship based on feed conversion ratio. Feed cost per kilogram of gain is equal to diet cost per kilogram multiplied by FCR. Accordingly, the break-even relative diet price was calculated as the FCR of the commercial control divided by the FCR of each fermented-sago diet. This partial analysis does not include fermentation, drying, labour, transport, quality-assurance or capital costs and should not be interpreted as proof of commercial savings. Actual least-cost feed formulation should consider current ingredient prices while meeting specified nutrient requirements (Pesti & Choct, 2023). The performance-adjusted break-even diet prices relative to the commercial positive control are summarised in Table 5.

Table 5
Performance-adjusted break-even diet price relative to the commercial positive control

Diet	FCR	Break-even price (% of PC price)	Interpretation
Positive control	1.97	100.0	Reference
10% fermented sago	1.96	100.5	Cost-neutral if diet price is no more than 0.5% above PC
20% fermented sago	1.93	102.1	Cost-neutral if diet price is no more than 2.1% above PC
30% fermented sago	2.13	92.5	Cost-neutral only if diet price is at least 7.5% below PC

Note. Break-even price (%) = FCR of positive control / FCR of treatment x 100. PC, positive control; FCR, feed conversion ratio

Limitations and Future Research

The findings were obtained under a single set of fermentation and feeding conditions. The study did not directly measure amino-acid composition, nutrient digestibility, enzyme activity, antinutritional factors, mycotoxin concentrations, gut histology, microbiota, meat sensory properties or consumer acceptance. The absence of clinical signs of mycotoxicosis is encouraging but does not replace chemical safety testing. Future work should validate the 20% inclusion level using larger replicated trials, current ingredient and processing costs, digestibility measurements and detailed meat-quality and safety assessments. Mixed-culture fermentation with yeast or other beneficial microorganisms may also be investigated to determine whether further nutritional improvement is achievable.

CONCLUSION

Solid-state fermentation using *Aspergillus niger* successfully enhanced the nutritional value of sago pith, demonstrating its potential as an alternative feed ingredient for broiler production. This study highlights the value of utilising locally available resources to reduce dependence on imported feed ingredients while supporting improved feed security and more sustainable poultry production systems. Further research should focus on evaluating the effects of fermented sago on sensory properties such as taste and texture, as well as investigating intestinal histology and gut microbiota characterisation to better understand its influence on broiler health and overall production performance.

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Food and Feeding Habits of Wild *Pangasius nasutus* (Bleeker 1863) in Jerantut District of Pahang River Mainstream, Malaysia

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ABSTRACT

Despite the ecological and commercial importance of *Pangasius nasutus*, limited information is available related to the feeding habits of the species in the natural river systems. This study was conducted to provide preliminary information on the food and feeding habits of wild *P. nasutus* in Jerantut District of Pahang River mainstream, Malaysia. A combination of frequency of occurrence and numerical methods was used. The length (cm) and weight (g) of all samples were measured, and their stomach contents were recorded. The differences in diet composition were compared between different total length size groups. A total of 265 individuals were collected from January 2022 to March 2023. Food items of *P. nasutus* revealed omnivorous feeding habits, although they consumed more animal-based foods than plant-based foods. Based on frequency of occurrence (% F), avian carcasses were the major food items that occurred with 38.92%. This was followed by *Corbicula fluminea* (29.34%), plant debris (21.56%), unidentified animal remains (15.57%), decapods (11.38%), Osteichthyes (10.18%), *Ficus* sp. fruits (9.58%), *Elaeis* sp. fruits (7.19%), sand grains (2.40%) and unidentified plant materials (0.60%). However, based on percentage prey abundance (%N), *C. fluminea* was the most abundant (86.11%). This was followed by avian carcasses (3.49%), plant debris (2.63%), Osteichthyes (2.36%), *Elaeis* sp. fruits (1.45%), unidentified animal remains (1.40%), decapods (1.29%), *Ficus* sp. fruits (0.91%), sand grains (0.32%) and unidentified plant materials (0.05%).

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Findings indicated that ontogenetic resource partitioning also played an important role in the coexistence of *P. nasutus* populations in the Pahang River, Malaysia.

Keywords: Feeding habits, ontogenetic resource partitioning, Pahang River, *Pangasius nasutus*, stomach content analysis

INTRODUCTION

The food and feeding habits of fish have always been an important area of research, especially in the studies of fisheries science and aquaculture. Knowledge of fish diets provides insight into a species' biology, including its condition, energy intake, and reproduction. In a broader sense, knowing what a fish species consumes can help fisheries scientists and managers to understand the trophic relationships, population dynamics and community dynamics within the ecosystem of the species, which enables comparisons between other systems (Amundsen & Sánchez-Hernández, 2019).

Pangasius nasutus, a species from the Pangasiidae family, can be found in the Perak and Pahang River basins of Peninsular Malaysia, and other riverine systems in Sumatra and Borneo (IUCN, 2020). In Malaysia, it is an important fish source and one of the most sought-after and preferred edible freshwater fish species. The price can be up to RM160 (USD 40) per kilogram. At all times, this species was classified as an omnivore that feeds on benthic invertebrates, seeds and fish (Gustiano & Pouyaud, 2005). However, there is a lack of empirical data on their diet and feeding habits. Furthermore, there is no solid information about their ecology, population size, and the effects of anthropogenic threats to the status of *P. nasutus* in the wild (Vidthayanon & Ng, 2020).

According to the IUCN Red List of Threatened Species, *P. nasutus* was assessed in 2019 and listed as “least concern”, stating its population trend to be stable (IUCN, 2020). However, a study by Jaafar et al. (2023) indicated that this species in the Pahang River is vulnerable to overfishing due to its slow growth rate. Therefore, investigating its food and feeding habits could help address existing research gaps, particularly regarding its ecological role within its habitat. Fish are both predators and prey embedded in complex trophic networks, and understanding the food web interactions through their dietary patterns is essential in developing harvesting strategies in maintaining fish stocks at a sustainable level (Traugott et al., 2020). This is becoming more important as world consumption of aquatic animal foods has increased significantly in recent years and will rise continually, with an average annual growth rate of 3.0 percent between 1961 and 2021, outpacing the annual population growth rate of 1.6 percent (Food and Agriculture Organization [FAO], 2024).

In Malaysia, several studies have been done related to food and feeding habits of fish species (Ara et al., 2016; Azfar et al., 2015; Azim et al., 2021; Hamid et al., 2015; Intan-

Faraha et al., 2020; Mustafa-Kamal et al., 2012). Malaysia is rich with different types of aquatic ecosystems, which provide high diversity of fishes that are commercially important, especially to the local communities. These high-value species are cultured for aquaculture production in the country. Despite this, scientific information on the food and feeding habits of *P. nasutus* is very limited and has only been shared verbally among the local fishermen.

Therefore, this study aimed to determine the food and feeding habits of wild *P. nasutus* in Jerantut District of Pahang River mainstream, Malaysia. To achieve the aim, two objectives were developed: first, to determine the stomach contents of *P. nasutus* and second, to compare differences in the diet composition of *P. nasutus* in the study area. This study will contribute to a better understanding of the feeding ecology and dietary composition of *P. nasutus* in the Pahang River, Malaysia. The findings will provide baseline information on the stomach contents and dietary variation of the species, which can improve knowledge about its ecological role, feeding preferences, and habitat utilisation in the wild. In addition, the study may support future fisheries management, conservation planning, and sustainable exploitation of the species, particularly in the riverine systems of the Pahang River, Malaysia.

METHODOLOGY

Site Description

Pahang River is the longest river in Peninsular Malaysia, with a length of 459 kilometres, and its upstream section of the river system is located in the Main Range of Titiwangsa (Lun et al., 2011). It is located in the State of Pahang, which is also the largest state in Peninsular Malaysia. The Pahang River Basin lies between latitude N 2° 48' 45" and N 3° 40' 24" and between longitude E 101° 16' 31" and E 103° 29' 34". It has two main tributaries named the Tembeling River and the Jelai River, which meet at the confluence of Kuala Tembeling.

The Pahang River plays an important role as a drainage system to drain excess water from the river basin to the South China Sea during the northeast monsoon season (Lun et al., 2011). In general, the annual rainfall of the basin was 2,170 mm. However, during the northeast monsoon season between October and January, the amount of rainfall reach to the peak and flooding in the lowland areas occurs frequently due to overflowing from the Pahang River (Lun et al., 2011).

Study Location and Duration

This study was conducted at a section of the Pahang River which is located in the Jerantut district of Pahang (Figure 1). The section was only a small part of the whole stretch of the Pahang River, with a length of about 40 kilometres. This section was selected as it was believed to be the district where *P. nasutus* are the most abundant. This was supported by

the findings of Jaafar (2024), which reported that Jerantut recorded the highest number of *P. nasutus* during their study period between 2021 and 2022. Furthermore, due to limitations in sampling resources such as time and manpower, it is justifiable that only the Jerantut district was explored as the study location.



Figure 1. Map of Jerantut District, Pahang River, Pahang, Malaysia (Google, 2023)

This study was conducted every month from January 2022 to March 2023 for five days per week each month. In total, the one-year and three-month study period was conducted to observe the monthly and seasonal patterns of food and feeding habits of *P. nasutus*.

Data Collection and Sampling

The main data collected for this study were the number of fish, length of fish (cm), weight of fish (g), weight of stomach, and stomach content data such as the number and type of food items consumed by *P. nasutus*.

Collection of fish samples was conducted using traditional fishing methods employed by local fishermen. This approach enabled efficient and practical sampling within the natural riverine environment and is considered suitable for feeding habit studies, as it reflects the species naturally captured in local fisheries. Although some selectivity toward certain size classes may occur, the method remains widely acceptable for dietary analysis because it provides representative specimens from the existing population in the study area. Similar approaches have been widely applied in previous dietary studies of freshwater and marine fishes (Zacharia & Abdurahiman, 2004).

An average of 20 samples in total were separated, weighed, and collected from the fishermen for each month. Each individual was wrapped in plastic and immediately frozen before being taken to the Aquatic Biology and Ecology laboratory located in the Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.

Laboratory Analysis of Samples

In the laboratory, all fish were defrosted to air temperature, and their total weight (g) was measured using a digital balance for each individual (Figure 2). Later, the fish were placed on a measuring board for the total length (cm) measurement (Figure 3). The total length of the fish was recorded, measured from the tip of the snout to the tip of the tail of the fish.



Figure 2. P. nasutus weighed using a digital balance



Figure 3. P. nasutus placed on a measuring board

Subsequently, scissors were used to cut open the ventral side of the fish from the anus all the way up to the gills. All the internal organs were removed by cutting at the upper part of the oesophagus to the anal opening. The stomach was removed from the other internal organs, beginning from the upper end of the oesophagus to the pylorus before the upper end of the fish intestine. Each stomach was then preserved in bottles with 10% formalin solution for further analysis.

For analysis of stomach contents, each stomach was dried using filter paper before the total stomach weight (g) was measured using a digital balance. The stomachs were cut using fine dissecting scissors, and all the items in the stomach were emptied into petri dishes, counted and identified visually (Figure 4). The items were then placed under a Leica compound microscope with 10× to 40× magnification for identification. After emptying the contents, the empty stomachs were weighed (g) using a digital balance.

All the contents of the stomachs were counted and identified to the lowest taxonomic level possible. Identification of the molluscs was performed using the taxonomic key provided by McMahon & Bogan (2001). Prey items that lacked identifiable diagnostic structures due to being highly digested were classified as unidentified animal remains, plant debris, and unidentified plant materials. Finally, the food items were preserved in bottles of 70% denatured ethyl alcohol and were labelled accordingly.

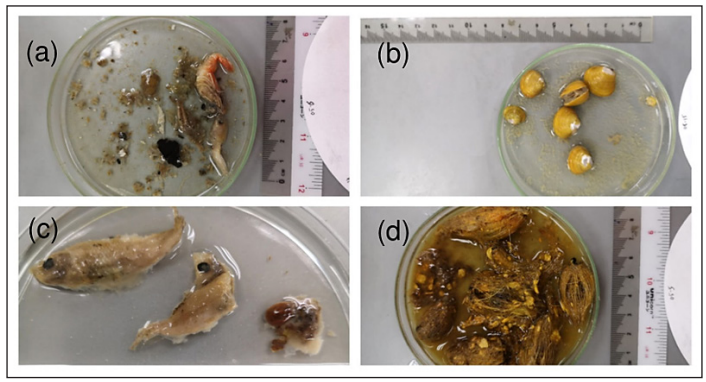


Figure 4. Examples of food items found in the stomach of *P. nasutus:* (a) unidentified decapoda or shrimps; (b) mollusc; (c) unidentified fish; (d) oil palm fruits (*Elaeis* sp.)

Frequency of Occurrence (%F)

The occurrence method was used to quantify the food items that occurred in the fish stomachs. The individual stomach contents were grouped into food categories, and the number of stomachs that contained one or more individuals of each category was recorded (Hyslop, 1980). Then, the numbers were expressed as a percentage of all stomachs with food. The equation for calculation of frequency of occurrence (f_i) is shown in Equation 1:

$$f_i = \frac{N_i}{N} \qquad [1]$$

where N_i is the number of fish with prey category i in their stomach, and N is the total number of fish with stomach contents (Amundsen & Sánchez-Hernández, 2019). Differences in digestion rates between hard-bodied prey (e.g., molluscs) and soft-bodied prey may influence detectability in stomach contents and may bias frequency-based estimates of diet composition; hence, the relative importance of some prey categories should therefore be interpreted with caution.

Percentage Prey Abundance (%N)

Apart from the occurrence method, the numerical method is also used to provide information on the amount of food consumed by *P. nasutus* for each category. This is done by recording the total number of individuals in each prey category and then expressed as a percentage of the total individuals in all prey categories (Hyslop, 1980). This gives the percentage prey abundance. The equation for calculation of percentage prey abundance (p_i) is shown in Equation 2:

$$p_i = \frac{\Sigma S_i}{\Sigma S_T} \quad [2]$$

where S_i is the amount of stomach contents for prey category i, and S_T is the total amount of prey present in each stomach for all categories. (Amundsen & Sánchez-Hernández, 2019).

Ontogenetic Diet Shifts

Differences in diet composition of *P. nasutus* were compared between body sizes to provide information on the ontogenetic diet shift among fishes. The fish samples were grouped into four total length size classes, which are 35.6 to 40.5 cm, 40.6 to 45.5 cm, 45.6 to 50.5 cm, and 50.6 to 55.5 cm. Later, differences in percentage prey occurrence and percentage prey abundance were compared. This interval width was selected to balance statistical robustness and ensure adequate sample sizes within each group. The selected range captured over 98% of the individuals sampled. Alternative size classes were explored during preliminary analysis but resulted in highly uneven sample distributions and were therefore not retained.

Statistical Analysis

The total number of fish caught during the study period was calculated, and all fish were categorised by size classes based on their individual total length (cm). For each category, the number of stomachs with or without food was also recorded, and their percentage were calculated. All statistical analyses were conducted using IBM SPSS software version 26. Pearson's Chi-square, Kruskal-Wallis, and Mann-Whitney tests were utilised to test for significant differences between different diet compositions and different total length size classes of *P. nasutus*.

RESULTS AND DISCUSSION

Overall Diet Composition of *P. nasutus*

This study gives the first overview of the food and feeding habits of *P. nasutus* in Jerantut District of the Pahang River mainstream, Malaysia. The occurrence method

and numerical method were both used to provide preliminary data on diet composition of this species, as both methods were among the most rapid and cost-efficient methods used, where their percentages in one sample indicate homogeneity of feeding among the populations (Hyslop, 1980). A total of 265 fish individuals of *P. nasutus* were collected from the Pahang River during the study period from January 2022 to March 2023. Out of all the specimens examined, 98 individual fish (36.98%) had empty stomachs, while 167 individual fish (63.02%) had at least one food item that they consumed. There are several factors which may result in fish with empty stomachs, such as differences in gastric evacuation rates, environmental conditions, or sampling time relative to feeding cycles (Vinson & Angradi, 2011). Further studies can be done to evaluate these factors which cause variations in the percentage of empty stomachs in fish.

Moreover, Pangasiid catfishes are often reported to exhibit nocturnal feeding behaviour in riverine systems (Segaran et al., 2023). However, the present study relied on fish obtained from local fishermen, and the exact capture times of individual specimens were not recorded. Therefore, it was not possible to evaluate diel feeding patterns or determine whether the observed diet composition reflects nocturnal feeding activities. Future studies incorporating controlled sampling with recorded capture times would provide further insight into potential diel feeding cycles of *P. nasutus*.

Overall Percentage Prey Abundance (%N) of *P. nasutus*

A total of 1,864 prey items were found and counted from the stomachs of all fishes during the study period from January 2022 to March 2023. *Corbicula luminea* (etak) (86.11%) were the most abundant, followed by avian carcasses (3.49%), plant debris (2.63%), Osteichthyes (2.36%), *Elaeis* sp. fruits (oil palm seed) (1.45%), unidentified animal remains (1.39%), decapods (1.29%), *Ficus* sp. fruits (0.91%), sand grains (0.32%) and unidentified plant materials (0.05%) (Figure 5). These results did not account for the sizes of the food categories, which had major differences; for example, *C. luminea* usually occurred in large numbers in a single sample, while avian carcasses were counted as a single food item that already made up the whole contents of a single stomach. One of the fish alone consumed a number of 379 *C. luminea*, which already made up 20.33% of all food items in terms of %N. The high %N of *C. luminea* may reflect high abundance of this species in the Pahang River. However, further studies are required, as studies related to the species were also conducted in other states in Malaysia, such as in Kelantan, Sabah and Sarawak (Aweng et al., 2021, 2022). The high numerical abundance of *C. luminea* may also be influenced by shell persistence in stomach contents, which could lead to overrepresentation in numerical counts compared to non-shelled prey items.

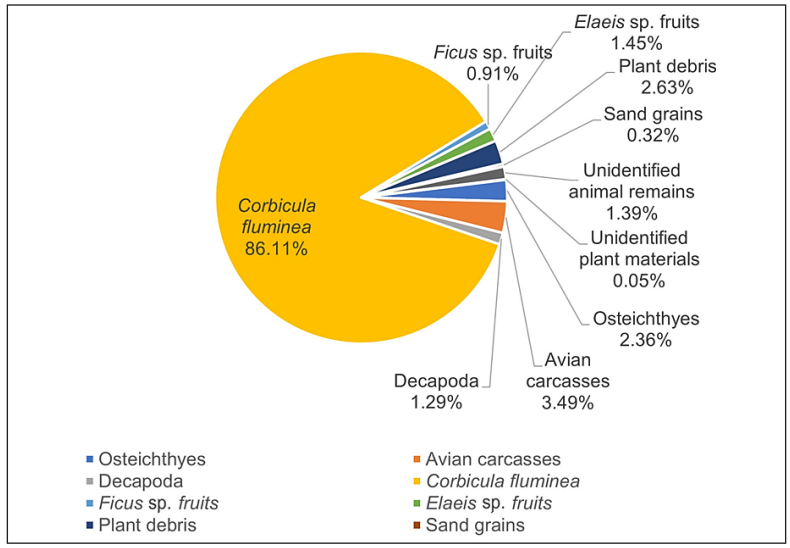


Figure 5. Percentage prey abundance (%N) of *P. nasutus* during the whole study period

Originally distributed in Asian waters, *C. fluminea* was reported as an invasive species in American and European freshwater habitats, as it can grow rapidly and has high fecundity, with tolerance to variable water conditions (Sousa et al., 2008). The dominance of this bivalve in the diet may reflect broader ecosystem-level changes associated with its proliferation, which can alter benthic community structure and nutrient cycling in freshwater systems (Sousa et al., 2008). In Malaysia, *C. fluminea* is a famous delicacy, especially in Kelantan state where it has always been harvested by the locals in Pergau Lake (Rak et al., 2022). Being a benthic feeder, *P. nasutus* would encounter the prey frequently, making it a readily available food source. In river systems where *C. fluminea* is absent or rare, the diet composition of *P. nasutus* may differ substantially depending on the availability of alternative benthic prey.

The reliance on *C. fluminea* could reflect resource partitioning, allowing coexistence with other fish species in the studied area. However, the dominance of *C. fluminea* in the diet of the fishes reflects the changes that occurred in the food web in the studied area, which remain to be fully explored as it may have contributed to the changes in fish abundance (Feyrer et al., 2003). Furthermore, the low representation of bony fish (2.36%) and decapods (1.29%) in the diet of *P. nasutus* indicated that the species may occupy a distinct trophic niche to avoid competition with sympatric piscivores or decapod specialists. The *P. nasutus* in the Kapuas River consumed prey items such as prawns, terrestrial insects and snails, while molluscs were more predominant in the stomach contents of *P. conchophilus* than any other Pangasius species in the river (Roberts & Vidthayanon, 1991). For unidentified animal remains (1.39%), plant debris (2.63%) and unidentified plant materials (0.05%),

these were present in highly digested forms, and the structures are no longer identifiable. It remains uncertain whether plant debris was intentionally consumed or incidentally ingested during benthic feeding activities.

Overall Frequency of Occurrence (%F) of Prey Items of *P. nasutus*

The results revealed that 65 out of 167 fishes (38.92%) consumed avian carcasses, and this was the highest percentage of food item that occurred in the stomachs of *P. nasutus* in the study area. This was followed by *C. fluminea* (29.34%), plant debris (21.56%), unidentified animal remains (15.57%), decapods (11.38%), Osteichthyes (10.18%), *Ficus* sp. fruits (9.58%), *Elaeis* sp. fruits (7.19%), sand grains (2.40%) and unidentified plant materials (0.60%) (Table 1).

The occurrence of "avian carcasses" is an unexpected and ecologically significant finding, which contradicts typical dietary patterns observed in many benthic catfish species. They are often described as omnivores or detritivores which focus on invertebrates, molluscs, plant matter, and small fishes (Haque et al., 2022; Haubrock et al., 2018; Khan, 1988; Rao, 2017; Roberts & Vidthayanon, 1991). Avian carcasses may represent a seasonally or spatially abundant food source, particularly in river systems that intersect with bird migration routes or nesting sites, such as Tekam Forest Reserve situated in the Jerantut District of Pahang, which serves as a habitat for many bird species (Hassan, 2017). Moreover, this occurrence also reflects anthropogenic subsidies such as poultry waste disposal. This study does not quantify the source of the avian carcasses, either from the contribution of natural or anthropogenic inputs cannot be determined and requires further investigation. The presence of avian remains may reflect opportunistic scavenging behaviour rather than targeted predation.

Table 1
Percentage frequency of occurrence (%F) of prey items of P. nasutus *during the study period from January 2022 to March 2023*

Prey items	No. of Fish Found with Prey Items (n)	%F
Avian carcasses	65	38.92
Corbicula fluminea	49	29.34
Plant debris	36	21.56
Unidentified animal remains	26	15.57
Decapoda	19	11.38
Osteichthyes	17	10.18
Ficus sp. fruits	16	9.58
Elaeis sp. fruits	12	7.19
Sand grains	4	2.40
Unidentified plant materials	1	0.60

Although the frequency of occurrence of avian carcasses (38.92%) appeared slightly higher than that of *C. fluminea* (29.34%), statistical analysis using the Chi-square test revealed no significant difference between the two ($X^2 = 3.41$, $p > 0.05$). The result indicates that both prey categories were consumed by *P. nasutus* with relatively similar frequency. This may suggest that both preys play a comparable, though possibly minor, role in the diet, potentially reflecting opportunistic feeding behaviour. Avian remains (e.g., feathers and bones) and mollusc shells may persist longer in the stomach compared to softer prey such as decapods or plant matter, potentially expanding their perceived occurrence (Buckland et al., 2017; Hyslop, 1980).

Other than that, the occurrence of bony fishes in the stomachs of *P. nasutus* indicated that the species is opportunistic and flexible in its feeding habits. The low occurrence and abundance of bony fishes consumed by *P. nasutus* does not define the species as piscivorous. Other than the *Ficus* sp. fruits, *P. nasutus* was also known to consume *Elaeis* sp. fruits. Most of the other plant-based food sources found were in small pieces, and they were classified as plant debris. It was unclear whether plant debris was ingested accidentally or was selected by the fishes during the study period. The low occurrence and abundance of sand grains may indicate that *P. nasutus* has a benthic foraging behaviour, possibly while feeding within the riverbed area, possibly when consuming *C. fluminea* (Admassu et al., 2015; Hamid et al., 2015). Sandy substrates produce a yellow colour to the shells of *C. fluminea* (Aweng et al., 2022). The occurrence of animal-based and plant-based foods in the diet of *P. nasutus* determined that the species is an omnivore fish.

Ontogenetic Variation of Food Consumption of *P. nasutus*

All the fish caught were grouped into different size groups based on their total length (cm). There were four size groups established, including from 35.6 cm to 40.5 cm, 40.6 cm to 45.5 cm, 45.6 cm to 50.5 cm, and 50.6 cm to 55.5 cm (Figure 6). A total of 121 individual fish had a total length ranging from 45.6 cm to 50.5 cm, the highest among all size groups. The proportion of empty stomachs was the highest in the size group of 50.6 cm to 55.5 cm (58.82%), while the lowest proportion of empty stomachs was in the size group of 35.6 cm to 40.5 cm (11.43%).

The observed increase in the percentage of empty stomachs, as fish size increases, may be due to changes in feeding frequency of the fish. The smaller fish require more frequent feedings to meet their metabolic demands compared to the larger fish. As they grow in size, they are able to consume larger prey or more food during each feeding session, thus requiring fewer feeding sessions per day. Vinson and Angradi (2011) revealed that the percentage of fish with empty stomachs was positively correlated to the total length of fish at maturity.

Ontogenetic Variation of %N of Prey Items of *P. nasutus* by Total Length (cm)

Overall, *C. fluminea* was the most abundant prey observed (%N) in all length size groups, although it showed different results in different size groups (Figure 6). In the 35.6 cm to 40.5 cm group, %N was 96.41%, while in the 50.6 cm to 55.5 cm group, %N was only 31.25%. Sample sizes varied across size groups, ranging from 14 to 60 individuals. Due to this imbalance and non-normal distribution of prey numbers, a Kruskal-Wallis test was implemented to reduce sensitivity to unequal variances and non-normal distributions, and to test differences in numerical abundance. The results revealed a significant difference between the groups ($H = 24.66$, $p < 0.05$), while the post-hoc pairwise comparisons showed that fishes in the 35.6 cm to 40.5 cm group had more *C. fluminea*, significantly difference ($p < 0.05$) when compared to the other three size groups. In terms of abundance, dietary breadth increases as size increases, as *P. nasutus* shifted its diet from *C. fluminea* to a diverse range of food items. This shows that niche or resource partitioning occurs to reduce dietary overlap among small and large individuals and possibly reduce interspecific competition for food between and within species. High dietary diversity in larger individuals may reflect both genuine expansion of dietary breadth and greater cumulative prey intake, due to a larger stomach capacity.

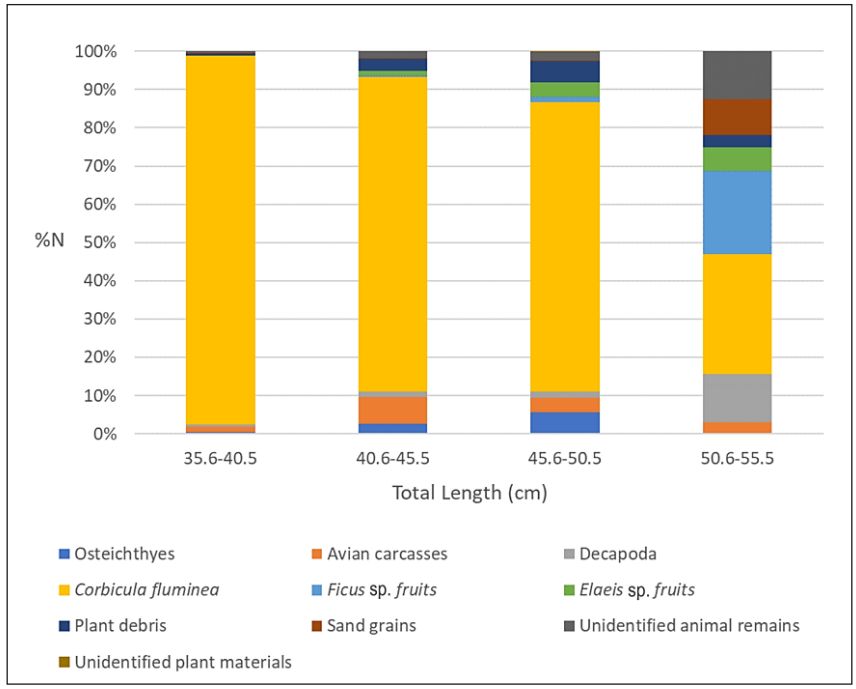


Figure 6. Percentage prey abundance (%N) of *P. nasutus* of different size groups (total length in cm)

It was observed that *C. fluminea* was an important food source of *P. nasutus*, although the percentage of prey abundance (%N) decreased as fish increased in length. In contrast with other fish species, it is not uncommon that smaller fish individuals were observed to consume small planktonic invertebrates, particularly among the juveniles (Intan-Faraha et al., 2020; Pereira et al., 2015). The current study revealed that smaller *P. nasutus* focused on molluscs as their main prey items, while the larger individuals consumed more decapods (mostly appendages). This may also be due to larger fish individuals foraging in different microhabitats within the river areas where they encounter different prey assemblages (Sánchez-Hernández et al., 2019). Generally, the results above show that *P. nasutus* can be classified under benthopelagic fish feeding types.

Ficus sp. And *Elaeis* sp. Fruits were not found in the stomachs of the smallest size group (35.5cm to 40.5cm) and were observed in abundance in the bigger size groups. Fish in the largest size group (50.6cm to 55.5cm) had significantly higher *Ficus* sp. Fruits when compared to the other size groups ($H = 25.43$, $p < 0.05$). However, the numerical abundance of *Elaeis* sp. Fruits did not differ significantly between different size groups ($H = 5.81$, $p > 0.05$). Plant debris also increased numerically, which may indicate that *P. nasutus* shift their diet from a carnivorous diet to an omnivorous diet, which includes plant food items. This shift in the diet was common among fishes in various studies and may be driven by both ecological and physiological factors. German et al. (2014) discovered that *Phytichthys chirus* consumed larger proportions of algae as they increased in size, which exhibited changes in gut length and an increase in digestive enzyme activities.

Ontogenetic Variation of %F of prey items of *P. nasutus* based on Total Length (cm)

The differences in the highest percentage of prey occurrence and prey abundance, based on the total length size groups, are presented in Figure 6 and Table 2. Other than percentage prey abundance, C. fluminea (61.29%) also had the highest percentage prey occurrence in the size group of 35.6 cm to 40.5 cm, with a chi-square test showing a significant difference ($X2 = 25.75$, $p < 0.05$). Avian carcasses occurred the most in the stomachs of fish of the size group of 40.6 cm to 45.5 cm (51.67%), and it was significant ($X2 = 10.70$, $p < 0.05$), while Ficus sp. Fruits (42.86%) occurred the most in the size group of 50.6 cm to 55.5 cm, and it was significance ($X2 = 25.16$, $p < 0.05$).

Table 2
Frequency of occurrence (% F) of prey items of P. nasutus *for each size group (total length)*

Prey Items	%F			
	Size Groups (Total Length)			
	35.6 – 40.5 cm (n = 31)	40.6 – 45.5 cm (n = 60)	45.6 – 50.5 cm (n = 58)	50.6 – 55.5 cm (n = 14)
Corbicula fluminea	61.29	31.67	13.79	7.14
Avian carcasses	35.48	51.67	34.48	7.14
Ficus sp. Fruits	0.00	3.33	12.07	42.86
Plant debris	12.90	20.00	31.04	7.14
Decapoda	12.90	10.00	12.07	14.29
Unidentified animal remains	9.68	13.33	18.97	28.57
Osteichthyes	9.68	11.67	12.07	0.00
Sand grains	3.23	1.67	1.72	7.14
Elaeis sp. Fruits	0.00	5.00	12.07	14.29
Unidentified plant materials	0.00	0.00	1.72	0.00

Although prey size was not measured in this study, visual observations showed that prey size increased with predator size, which was shown by the increase in occurrence of *Ficus* sp. As *P. nasutus* increased in size. Riparian fig trees are commonly found along a river system, making them accessible to surface or mid-water feeder fishes (Pothasin et al., 2014). This food item was not found in fishes of the smaller group (35.6 cm to 40.5 cm), which may be due to gape size, where larger individuals with greater gape size and swimming capacity may be more efficient in exploiting the *Ficus* sp. The ontogenetic variations may be related to the changes in types of prey consumed in different habitats. Further studies can be done in other areas of the Pahang River to compare the differences in prey items consumed, related to the mouth size, and habitat tilizedon of the *P. nasutus* in a river system.

Furthermore, the decrease in occurrence of *C. fluminea* in the stomachs of *P. nasutus* as they increase in length shows that some individuals exhibited selective feeding behaviour and had a strong preference for *C. fluminea*, while others, in general, exhibited generalist feeding behaviours. The introduction of avian carcasses due to human activities may influence changes in the diet composition of *P. nasutus*, as found in the size group of 40.6 to 45.5 cm, where they fed on larger prey as a preference. Long-term dietary studies with adequate sample size across all ontogenetic stages can reveal a true dietary pattern and interannual variations of fish species (Sánchez-Hernández et al., 2022).

The difference in diet composition between fish sizes provides a clearer view of both the biological and ecological aspects of *P. nasutus*. Resource partitioning is observed between fish of different sizes as they exhibit ontogenetic dietary shifts. Smaller fish

primarily consumed *C. fluminea*, while larger fish consumed a more diverse diet, including decapods, plant debris, and fruits. This shift suggested potential ontogenetic resource partitioning, which may reduce dietary overlap among size classes. The dietary flexibility of *P. nasutus* illustrates that the species has potential as a bioindicator for monitoring trophic conditions in river systems, although additional ecological studies would be required to confirm this role. This trophic information should inform multi-scale management approaches that integrate habitat conservation and fisheries regulation to ensure the long-term sustainability of wild *P. nasutus* populations in the Pahang River. However, feeding ecology alone is insufficient to reassess the conservation status of *P. nasutus*. Additional analyses would be required to establish stronger links between trophic ecology and conservation assessments, including long-term population monitoring, evaluation of habitat quality, assessment of prey availability, and comparisons across multiple river systems.

One of the limitations faced when conducting this study was the unevenness of the sample size. Other than that, the fish samples collected were constrained to one location of the whole stretch of the Pahang River. Further studies should be conducted on other parts of the Pahang River to give a broader view on the diet of *P. nasutus*. As the sampling location increases, possible differences in food items occurring in the stomachs of the species may provide information about relationships of food, feeding habits and different habitats tilized by the fishes. Although prey size appeared to increase with predator size based on visual observations, quantitative morphometric measurements were not performed. Therefore, conclusions regarding gape limitation should be interpreted cautiously. Other than that, soft-bodied prey items may be affected because of faster digestion and reduced detectability during microscopic examination.

Future studies should also implement DNA-based techniques, which are useful to improve the accuracy of results obtained from traditional methods, such as improving identification of highly digested prey items and reducing the proportion of unidentified materials. Finally, environmental parameters that could influence fish behaviour and its diet, such as water temperature, pH, dissolved oxygen, flow rates, and habitat complexity, should also be accounted for in future studies. Sampling of prey in the river system would also provide a clearer view of food availability and distribution, which in turn impacts the feeding success of *P. nasutus*. Information gained in this study could help to improve aquaculture practices of the species and conserve riparian vegetation such as *Ficus* species along the Pahang River to ensure species sustainability in the natural habitat. As the study was conducted over a period of one year and three months, the results represent a snapshot of feeding patterns within this time-frame. Longer-term studies would be required to determine whether the observed dietary patterns remain consistent across years.

CONCLUSION

This study provides a critical understanding towards the feeding habits of *P. nasutus* in the Pahang River, Malaysia. Results obtained provide insight into the abundance of *C. fluminea* as the main food item present in the stomach of *P. nasutus*. The occurrence of animal-based and plant-based food items in the diet of *P. nasutus* confirmed the species as omnivorous fishes with opportunistic feeding behaviours, indicating a diverse and wide range of diet for the species. The high occurrence of avian carcasses was an interesting finding, not as a natural food source within the aquatic environment, but likely associated with human activities occurring in the surrounding areas. The dietary composition across ontogenetic stages indicated that *P. nasutus* shifts from predominantly animal-based food items to more plant-based resources as body size increases, suggesting that different size classes have distinct nutritional and energetic requirements throughout development. The findings should enhance all efforts made by the fisheries managers to ensure sustainable management planning and conservation programs for *P. nasutus* in the Pahang River, Malaysia.

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Assessing the Impact of Land Use on Arbuscular Mycorrhizal Root Colonisation Rate, Phosphorus and Nitrogen Uptake in Ife Bimpe Cowpea

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ABSTRACT

Soil characteristics, microbial ecology and organic additions influence soil fertility and plant productivity. However, the effects of land use and organic amendments on arbuscular mycorrhizal fungi (AMF), which enhance nutrient uptake and tolerance to soil stress, remain insufficiently understood. This study examined the effects of land use and organic amendments on AMF spore abundance, root colonisation and nutrient uptake. Soils from African Star Apple (ASAR), Cassava (CRR) and Bush Fallow (BFR) collected from farms behind Babcock University stadium, Nigeria, were analysed for pH, organic carbon, total nitrogen and available phosphorus. AMF spore abundance was assessed at 3-week intervals over 9 weeks following application of poultry manure (5 t ha^{-1}), cow dung (10 t ha^{-1}), and poultry manure plus cow dung (5 t ha^{-1} + 10 t ha^{-1}) before planting. Soil pH ranged from 7.00 (CRR) to 5.54 (BFR). ASAR had the highest organic carbon (5.46 g/kg) and total nitrogen (0.23%), while CRR had the highest phosphorus (4.727 mg/kg). CRR recorded the highest AMF root colonisation (48%) and Gigaspora spp. abundance, whereas ASAR had the highest total spore count (62%). Poultry manure increased root colonisation (44%) but reduced spore production, while cow dung maintained higher spore abundance (57%). Plant height and leaf development varied among land-use types, with ASAR soil supporting superior growth by Week

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9. AMF parameters were positively related to soil fertility indicators. Overall, cow dung promoted AMF spore abundance, whereas poultry manure facilitated root colonisation; CRR soils favoured colonisation, while ASAR soils favoured sporulation.

Keywords: Cow dung, *Gigaspora* spp., organic amendments, poultry manure, soil fertility indicators, soil origin, spore density, symbiotic fungi

INTRODUCTION

Phosphorus (P) and nitrogen (N) deficiencies are important constraints that severely limit crop productivity and threaten food security in tropical agroecosystems (Begum et al., 2019; Rillig et al., 2019). Arbuscular mycorrhizal fungi (AMF) create symbiotic relationships with plant roots that allow for the assimilation of these essential nutrients and so promote plant tolerance to environmental stress and enhance overall productivity (Augé et al., 2015; van der Heijden et al., 2015). AMF symbiosis is an active method for sustainable enhancement of crop yields on nutrient-poor soils with increasing global food demand and decreasing soil fertility (Wang et al., 2023).

Land use changes, notably the conversion of natural habitats such as forests into agricultural lands such as shrubby pasture-lands, have major implications for AMF diversity and function (Soka & Ritchie, 2018; Turrini et al., 2018). Such changes are generally associated with reduced AMF root colonisation and disrupted nutrient cycling, largely as a result of increased soil disruption and changes in vegetation structure (Davison et al., 2015; Trejo et al., 2016). While the effects of land use on AMF communities have been extensively investigated, there is a dearth of information about the consequences of these changes for AMF-plant interactions in different crop cultivars and agro-ecological zones, particularly in tropical soils which have been mostly neglected.

Addressing this knowledge gap is important as legumes, including cowpea (Vigna unguiculata), are important for food security and soil fertility through biological nitrogen fixation and nutrient cycling. The cowpea variety 'Ife Bimpe' is widely grown in the tropics and provides an excellent model to study these relationships under different land use systems. This study evaluated the effects of soils collected from different land-use types and organic amendments (poultry manure, cow dung, and their combination) on AMF spore abundance, root colonisation, soil nutrient availability, nutrient uptake, and cowpea productivity.

MATERIALS AND METHODS

Study Sampling Location and Experimental Set-up

The experiments were conducted in the screen-house of Babcock University, Ilishan-Remo, Ogun State, Nigeria, with a mean rainfall of 2400 mm at latitude 6.8932E and longitude

3.715N. Soil samples for this study were collected from different land use types, which include African star apple soil (ASAR), fallow bush soil (BFR), and cassava root soil (CRR) Figure 1. Sampling was conducted at a depth of 0-15 cm, at farms located behind Babcock University stadium, using a soil auger at 10 randomly selected points within each rhizosphere. Poultry manure (5 t ha^{-1}), cow dung (10 t ha^{-1}), and the combined treatment of poultry manure and cow dung (5 t ha^{-1} + 10 t ha^{-1}) were thoroughly mixed with the soil one week before planting to allow for decomposition and nutrient release prior to sowing. A 3x4 factorial experiment on a Completely Randomised Design (CRD), which was replicated three times, was used to set up the experiment as shown in Table 1.

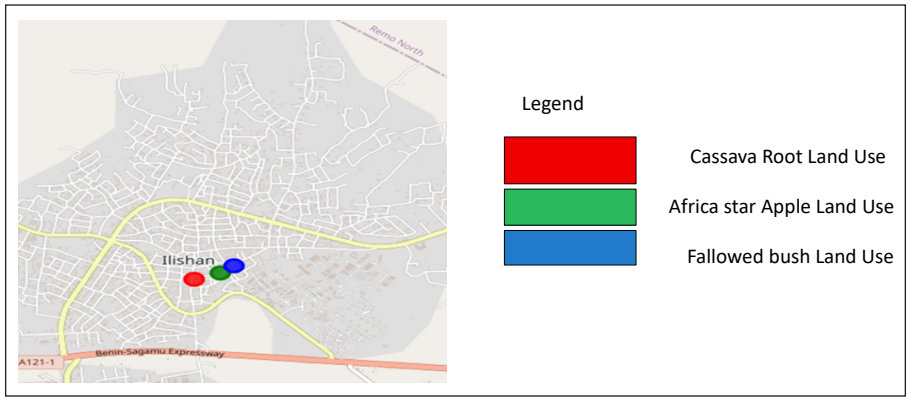


Figure 1. Satellite map of the study site behind Babcock University (Latitude: 6.8932° N, Longitude: 3.715° E), showing sampling locations for the three land use types: ASAR (African star apple), BFR (fallowed bush), and CRR (cassava root) plantations.
Source: Google Earth Pro satellite imagery (Google, 2025)

Table 1
Completely randomised design (CRD) layout of the experiment

Different Rhizosphere Soil Sources	B0 (None)	B1 (PM @ 5 t/ha)	B2 (CD @ 10 t/ha)	B3 (PM + CD Mixture)
ASAR	T1	T4	T7	T10
CRR	T2	T5	T8	T11
BFR	T3	T6	T9	T12

Note. Legend: ASAR = soil sampled from African star apple, BFR = soil sampled from Fallowed bush, CRR = soil sampled from Cassava root, B0 = No manure (sole AMF soil), B1 = Poultry Manure (PM) @ 5 t/ha, B2 = Cow Dung (CD) @ 10 t/ha, B3 = Mix of PM + CD @ 5 t/ha and 10 t/ha respectively

Organic Amendment Characterisation

The physicochemical properties of the organic amendments are presented in Table 2. Cow dung exhibited an alkaline pH, whereas poultry droppings showed near-neutral to

slightly alkaline characteristics. Poultry droppings had higher nitrogen, phosphorus, magnesium, and calcium contents compared with cow dung, while cow dung recorded a higher potassium content.

Table 2
Organic amendment

Properties	Poultry Manure	Cowdung
pH in H_2O	6.52	9.24
Total nitrogen (%)	4.41	5.03
Organic carbon (%)	35.50	23.78
Total P (%)	0.85	0.31
Ca (Cmol/kg)	3.56	0.71
Mg (Cmol/kg)	1.54	1.11
K (Cmol/kg)	0.53	3.13

Screenhouse Experiment

A five-kilogram (5 kg) portion of the soil from different treatment combinations was introduced into 7kg pots (7-litre bucket), perforated basally to facilitate drainage of any excess water. For Muriate of potash, straight fertiliser was applied at a standard rate of 30 kg/ha on each of the experimental pots. However, Nitrogen and phosphate fertilisers were not applied to observe the level of abundance of AMF spores, how they can colonise cowpea roots, their level of influence on phosphorus and nitrogen absorption by the cowpea crop, as well as their influence on different soil phosphorus and nitrogen availability. The soil was watered, and after a week, seven seeds of cowpea were planted, and it was thinned down to five seedlings at week two after planting.

Laboratory Analysis

Soil samples were air-dried and sieved in preparation for subsequent physical and chemical analyses. Particle size distribution was assessed using the hydrometer method (Agbenin, 1995; Bouyoucos, 1962), with a 5% Calgon solution as the dispersing agent. The organic carbon content was determined by the potassium dichromate method (Walkley & Black, 1934), where one gram of soil was mixed with $K_2Cr_2O_7$ and H_2SO_4, incubated, and then titrated with $FeSO_4$. Soil pH was measured using a pH meter in a 1:1 soil-to-water ratio (Thomas, 1996). Available phosphorus was extracted using Bray 1 solution (Murphy & Riley, 1962) by mixing one gram of soil with 7 mL of the Bray solution. Exchangeable bases were extracted with 1N ammonium acetate at pH 7 (Okalebo et al., 1993), and potassium (K) and sodium (Na) concentrations were quantified using flame photometry, while calcium (Ca) and magnesium (Mg) were analysed via Atomic Absorption Spectroscopy (AAS). Exchangeable acidity, comprising Al^{3+} and H^+, was determined by extraction with 1N KCl followed by titration (McLean, 1965). Total nitrogen content was quantified using the Kjeldahl digestion method (Bremner, 1965). Mycorrhizal propagule density and diversity were determined using wet sieving and decanting methods as described by Gerdemann and Nicolson (1963). AMF root colonisation was examined using the gridline intersect method according to Giovannetti and Mosse (1980).

The characteristics of the organic amendments were determined before application using the same analytical procedures described for the soil samples. The measured properties are presented in Table 2.

Data Collection and Statistical Analysis

Data were collected on plant height and number of leaves from 3 WAP, while from weeks 6 to 9, data were collected on root colonisation rate and phosphorus uptake. Nevertheless, from weeks 9 to 12, data were collected on biomass accumulation and yield (Dry shoot weight, pod weight, number of pods). Data collected were subjected to analysis of variance and correlation, while means were separated using the Duncan Multiple Range Test (DMRT) at 5%, and using Statistical Analysis Software 9.4. Graphs were plotted using the OriginPro 2024 software.

RESULTS AND DISCUSSION

Physical and Chemical Soil Properties of Various Land-Use Types

Table 3 shows the physical and chemical properties of soils collected from three different land-use types: African Star Apple soil (ASAR), Bush Fallow soil (BFR), and Cassava soil (CRR). The pH values indicated slightly acidic conditions in ASAR (5.56) and BFR (5.54), while the CRR soil was neutral (7.00). ASAR had the highest organic carbon (5.56 g/kg) and total nitrogen (1.25 g/kg), followed by BFR (3.59 g/kg OC; 0.74 g/kg N), with CRR having the lowest values (2.19 g/kg OC; 0.25 g/kg N). ASAR and BFR soils recorded limited concentrations of available phosphorus compared to CRR soil, with the highest P concentration (4.727 mg/kg). The improved nutrient availability and microbial activity observed in soil collected from the CRR land use system could be due to its near-neutral pH (Havlin & Heiniger, 2020; Weil & Brady, 2017), whereas the slightly acidic conditions in ASAR and BFR soils may have fixed the availability of phosphorus and micronutrients like zinc and iron, making them temporarily unavailable for immediate use. While ASAR soil's relatively higher organic carbon content may create a conducive environment for microbial activities and improve soil stability (Lal, 2020), the lower carbon levels in BFR and CRR soils may cause limitations to fertility, highlighting how vital organic amendments are in soil processes (Samson et al., 2024; Wiesmeier et al*.,* 2019). The higher nitrogen levels observed in ASAR soil highly indicate its comparative ability to support vegetative growth compared to BFR and CRR soils with critically low nitrogen content. This indicates that both BFR and CRR soils need nitrogen supplementation for optimal cowpea vegetative growth (Gadisa, 2020). Although CRR soil had higher available phosphorus, likely due to pH-mediated solubility (Braak et al., 2016), its low nitrogen and organic matter may negatively impact crop productivity. Thus, these findings reveal that ASAR soil requires limited input, whereas BFR and CRR soils may need integrated nutrient management to maintain adequate nutrient supply and support plant growth (Rahman et al., 2017).

Table 3
Physical and chemical properties of soil from different land use types

Sample	SOIL														
	pH (H2O)	**OC**	**N**	**Bray P**	**SAND**	**SILT**	**CLAY**	**Ca**	**Mg**	**K**	**Na**	**Zn**	**Cu**	**Mn**	**Fe**
	1:1	**(g/kg)**	**(g/kg)**	**(Mg/kg)**		**(g/kg)**				**(Cmol/kg)**			**(Mg/kg)**		
ASAR	5.56	5.56	1.25	1.540	690.0	90.0	220.0	1.4895	0.44	0.2835	0.064	0.03644	0.006	0.273	1.692
BFR	5.54	3.59	0.74	1.467	750.0	50.0	200.0	1.298	0.4235	0.5185	0.056	0.04145	0.004	0.346	1.571
CRR	7.00	2.19	0.25	4.727	790.0	50.0	160.0	1.966	0.4235	0.401	0.046	0.04485	0.006	0.2175	1.4425

Variation in Spore Abundance and Root Colonisation under Soils from Different Land-use Types and Organic Amendments Across Different Weeks

Differences in abundance of *Glomus* and *Gigaspora* spores and AMF root colonisation were observed across soils from different land-use types and organic amendment treatments (Table 4). At Week 3, soils from the bush fallow land-use type (BFR) recorded significantly higher *Glomus* spore counts (30.83), maintaining consistency in abundance up to Week 6 (48.92), which, however, declined to zero by Week 9. In contrast, soils from the African star apple land-use type (ASAR) maintained spore presence at Week 9 (1.17), indicating a more sustained AMF presence, suggesting that AMF propagules may persist more effectively under these soil conditions. The unamended control treatment had the highest *Glomus* counts at Weeks 3 and 6 (35.78 and 45.00), while poultry manure-amended soils (APM) retained spores (1.56) in Week 9. *Gigaspora* spores were initially more abundant in soils from the cassava land-use type (CRR) at Week 3 (3.08), while ASAR soils had the highest and most sustained sporulation at Weeks 6 and 9 (5.83 and 1.17, respectively).

Early *Gigaspora* sporulation was stimulated by cow dung (ACD, 4.11%) and poultry manure plus cow dung (APM&CD, 7.78%) at Week 3. However, only poultry manure-amended soils (APM) maintained spores at Week 9 (1.66). AMF root colonisation increased over time, with soils from the cassava and African Star Apple land-use types (CRR and ASAR) showing significantly higher colonisation (25.94% and 24.29%) than bush fallow soil (BFR; 21.73%) by Week 9. Unamended control treatments had the highest colonisation rates at all time points (7.40%, 22.79%, and 33.72%). These fertility patterns influenced mycorrhizal responses across the different land-use types and amendment treatments. In BFR soil, the increase and subsequent decline in Glomus spore abundance suggests a temporary AMF response, possibly associated with initial soil conditions (Guo et al., 2022). Conversely, soils from the African Star Apple land-use type and poultry manure-amended soils maintained Glomus spore production up to Week 9, which may reflect the sustained availability of nutrients and carbon from organic amendments that support soil microbial activity (Alzarhani et al., 2019; Enebe et al., 2025). The early and higher Gigaspora sporulation observed in soils from the cassava land-use type, together with its persistence in African Star Apple-derived soil, suggests favourable interactions between host plants and AMF communities under cowpea cultivation (Mukhongo et al., 2023). Among the organic amendment treatments, cow dung (ACD) and poultry manure combined with cow dung (APM & CD) enhanced early Gigaspora activity, while only APM maintained sporulation through Week 9, indicating a sustained effect of amendment application on AMF development (Zhang et al., 2020).

Table 4
Level of spore abundance and %amf root colonisation as influenced by soil from different land-use types and soil amendment across different weeks

Treatment	Glomus sppwk3	Glomus sppwk6	Glomus sppwk9	Gigaspora sppwk3	Gigaspora sppwk6	Gigaspora sppwk9	%amf root colonisedWK3	%amf root colonisedWK6	%amf root colonisedWK9
Soil									
ASAR	21.08±1.9b	36.17±2.3b	1.17±0.6a	1.92±1.0b	5.83±3.1a	1.17±0.6a	4.63±0.9b	16.64±0.7a	24.29±2.0a
BFR	30.83±4.2a	48.92±2.9a	0.00±0.0b	0.33±0.3c	3.33±1.2b	0.00±0.0b	6.13±0.5a	14.54±2.4b	21.73±2.7b
CRR	16.92±2.8c	24.75±2.3c	0.00±0.0b	3.08±1.7a	2.83±1.5b	0.00±0.0b	6.48±0.6a	16.69±2.1a	25.94±3.7a
Fertiliser									
Control	35.78±4.1a	45.00±4.9a	0.00±0.0b	0.44±0.4c	2.78±1.4c	0.00±0.0b	7.40±0.6a	22.79±1.3a	33.72±1.1a
ACD	16.33±2.7c	35.33±2.5b	0.00±0.0b	4.11±2.1a	5.44±1.8b	0.00±0.0b	5.76±0.6b	17.40±2.0b	29.18±3.0b
APM	25.22±3.1b	38.33±3.9b	1.56±0.8a	0.00±0.0c	0.00±0.0d	1.56±0.8a	5.39±b0.9c	12.30±1.3c	17.26±2.2c
APM&CD	14.44±1.0c	27.78±4.3c	0.00±0.0b	2.56±1.3b	7.78±4.0a	0.00±0.0b	4.43±0.8c	11.34±1.3c	15.79±1.7c
S*T	*	*	*	*	*	*	*	*	*

Note. Means with the same letter (s) in a column are not significantly different at the 5% level of probability by Duncan Multiple Range Test (DMRT); ns: not significant

The Interactive Effects of Land-use type and Organic Amendments on AMF Root Colonisation and Spore Abundance

Table 5 shows the effects of land-use type × organic amendment interaction on AMF colonisation and spore abundance. In the African Star Apple soil × control interaction (ASAR × Control), AMF root colonisation increased significantly from 7.94% at Week 3 to 32.39% at Week 9, exceeding the ASAR soil × poultry manure plus cow dung (APM & CD) interaction. The BFR soil× cow dung (ACD) interaction recorded a marked increase in colonisation from 3.70% to 40.01% by Week 9. Similarly, the CRR soil × Control interaction showed continuous colonisation, reaching 34.33% at Week 9. For Glomus spp., the CRR soil × Control interaction recorded the highest spore abundance at Week 3 (51.00 spores), followed by the ASAR soil × poultry manure (APM) interaction at Week 6 (45.33 spores). The BFR soil× ACD interaction recorded 27.33 spores at Week 9, indicating a delayed sporulation response. Gigaspora spp. showed higher sporulation under the ASAR soil × APM&CD interaction, with the highest value of 7.67 spores in Week 3. The BFR soil × ACD interaction also showed early sporulation, with 12.33 spores in Week 3 and 11.33 in Week 6, followed by a decline over time.

Root colonisation patterns further emphasised the influence of rhizosphere conditions on AMF development. ASAR and CRR soils recorded significantly higher AMF colonisation than BFR soil, with the unamended control treatment across the land-use types showing greater colonisation than amended treatments, highlighting the contribution of less disturbed and naturally regulated soil environments (Alguacil et al., 2017; Storer et al.,2017; Zhang et al., 2023). The higher colonisation observed in the ASAR soil × Control treatment interaction further supports the importance of stable conditions in promoting AMF symbiosis (Liu et al., 2020; Van Geel et al., 2022). In contrast, the gradual increase in colonisation under the BFR soil × ACD combination suggests that the slow nutrient release from cow dung may support progressive AMF establishment (Alguacil et al., 2017; Rillig et al., 2019). In CRR soil, the consistent colonisation observed in unamended control treatments further indicates compatibility between plant roots and native AMF communities (Wei et al., 2024).

Spore production patterns followed similar trends. The CRR soil × Control treatment interaction recorded the highest Glomus spore abundance, indicating suitable conditions for AMF multiplication. The ASAR soil × APM interaction also showed increased spore production, demonstrating the positive influence of poultry manure application (Alzarhani et al., 2019). The gradual but sustained sporulation observed in the BFR soil × ACD interaction corresponds with slower nutrient release during amendment decomposition (Storer et al., 2017). For Gigaspora, the ASAR soil × APM & CD interaction promoted higher early sporulation, suggesting combined amendment effects. However, the decline observed in BFR soil × ACD over time may reflect reduced nutrient availability as amendment resources were depleted (Mukhongo et al., 2023).

Table 5
Influence of land-use type × soil amendment interactions on AMF root colonisation and spore abundance

Soil	Fertiliser	%amf root colonisedWK3	%amf root colonisedWK6	%amf root colonisedWK9	Glomus sppwk3	Glomus sppwk6	Glomus sppwk9	Gigaspora sppwk3	Gigaspora sppwk6	Gigaspora sppwk9
ASAR	Control	7.94±0.3ab	18.86±0.6cd	32.39±1.5b	24.67±2.0bc	37.00±3.2cd	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
	ACD	7.05±0.1abc	15.23±2.3de	26.38±3.6c	17.00±1.2cd	36.33±1.5cd	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
	APM	2.14±0.01ef	16.76±0.8de	21.21±0.6d	28.67±1.2bc	45.33±1.2b	4.67±0.8a	0.00±0.0c	0.00±0.0d	4.67±0.9a
	APM&CD	1.40±0.03f	15.73±0.9de	17.18±1.8d	14.00±2.5e	26.00±1.2e	0.00±0.0b	7.67±1.3b	23.33±2.9a	0.00±0.0b
BFR	Control	8.36±0.4a	22.20±1.1bc	34.44±2.0b	31.67±2.2b	34.00±1.5d	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
	ACD	3.70±1.1def	24.20±1.6ab	40.01±0.8a	7.67±1.5f	27.33±2.7e	0.00±0.0b	12.33±2.0a	11.33±0.7b	0.00±0.0b
	APM	7.48±0.7abc	9.28±2.1gh	8.68±0.1e	13.67±1.5e	23.00±0.6e	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
	APM&CD	6.37±0.2abc	11.08±0.8gh	20.64±0.9d	14.67±1.8d	14.67±1.8f	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
CRR	Control	5.92 ±1.7bc	27.32±1.1a	34.33±2.7b	51.00±2.9a	64.00±2.5a	0.00±0.0b	1.33±1.3c	8.33±1.2bc	0.00±0.0b
	ACD	6.53±0.7abc	12.76±1.9ef	21.16±0.7d	24.33±c3.3d	42.33±2.3bc	0.00±0.0b	0.00±0.0c	5.00±2.7c	0.00±0.0b
	APM	6.54±0.4abc	10.88±0.6gh	21.90±0.7cd	33.33±2.6b	46.67±1.5b	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b
	APM&CD	5.52±0.7cd	7.22±0.72h	9.54±0.4e	14.67±1.8e	42.67±3.5bc	0.00±0.0b	0.00±0.0c	0.00±0.0d	0.00±0.0b

Note. Means with the same letter (s) in a column are not significantly different at the 5% level of probability by Duncan Multiple Range Test (DMRT)

Influence of Land-Use Type and Soil Amendments on Soil Available Phosphorus (P), Nitrogen (%N), and Plant Nutrient Uptake Over Weeks 3, 6, and 9

A significant variation in nutrient dynamics was observed among the land-use types and amendment treatments (Table 6). The soil from the African star apple land-use type (ASAR) recorded significantly higher available phosphorus (P) concentrations at Week 3 (55.22 mg/kg) and Week 6 (58.46 mg/kg), while the soil from the cassava land-use type (CRR) had the highest value at Week 9 (54.35 mg/kg). The soil from the bush fallow land-use type (BFR) had consistently lower P values throughout.

Among amended treatments, the combination of land-use types, poultry manure, and cow dung (APM&CD) resulted in the highest soil phosphorus concentrations, ranging from 79.25 to 78.07 mg/kg across sampling weeks. In terms of soil nitrogen (N), ASAR soil consistently recorded significantly higher values than CRR and BFR soils at all weeks.

The APM & CD amendment also significantly increased soil nitrogen levels relative to other treatments. By Week 9, ASAR soil showed the highest plant phosphorus percentage (%P = 0.29%) and nitrogen percentage (%N = 2.73%). The APM & CD treatment closely followed, with %P and %N values of 0.29% and 2.67%, respectively. These biological responses influenced nutrient dynamics. Elevated phosphorus in ASAR soil during the early weeks may be linked to soil-driven microbial solubilization (Alori et al., 2017), while CRR soil's increase by Week 9 likely reflects cumulative microbial and root activity (Richardson & Simpson, 2011). Interaction between APM & CD treatment steadily maintained phosphorus concentration, revealing a complementary role of organic amendments and AMF (Deguchi et al., 2012; Zhang et al., 2020). ASAR soil also consistently influenced higher nitrogen levels, which improved microbial transformation and nutrient cycling (Jamil et al., 2022). Likewise, APM & CD treatment interaction actively promoted nitrogen availability, reflecting microbial turnover and mineralisation (Merin et al., 2024). Higher plant %P and %N in ASAR soil and APM & CD treatments corroborates previous reports on improved nutrient uptake facilitated by AMF symbiosis (Liu et al., 2020; Xiang et al., 2020), affirming functional alignment between rhizosphere type, organic inputs, and AMF activity.

Interaction Effects of Land-use Type and Organic Amendment Combinations on Spore Density, Soil Nutrients, and Plant Phosphorus Uptake

This study significantly showed the impact of land-use type interactions with organic amendments on arbuscular mycorrhizal fungi (AMF) spore counts and soil nutrient dynamics, as shown in Table 7. The interaction between soil from African star apple land-use type (ASAR) and cow dung recorded the highest AMF spore counts, 134.33 and 268.67 spores at Weeks 3 and 6, respectively. This was significantly higher compared to the control unamended treatment (79.67 and 159.33 spores) and comparable to the ASAR soil-poultry manure interaction, which had 122.33 and 244.67 spore counts. The bush

Table 6

Influence of land-use type × soil amendment interactions on soil available phosphorus, total nitrogen, and plant nutrient uptake across growth periods

Treatment	AVAILABLE Pwk3	AVAILABLE Pwk6	AVAILABLE Pwk9	S%N WK3	S%N WK6	S%N WK9	plt%P wk6	plt%P wk9	plt%N wk6	plt%N wk9
Soil										
ASAR	55.22±5.7a	58.46±7.0a	48.01±9.5b	0.27±0.01a	0.27±0.01a	0.25±0.00a	0.23±0.01	0.29±0.02a	1.48±0.03	2.73±0.04a
BFR	39.16±7.9c	50.69±7.2b	37.87±8.0c	0.14±0.00b	0.14±0.00b	0.12±0.00b	0.25±0.01	0.25±0.02b	1.45±0.06	2.51±0.07b
CRR	51.89±7.9b	51.14±8.6b	54.35±8.3a	0.12±0.00c	0.13±0.01c	0.11±0.00c	0.25±0.01	0.25±0.02b	1.40±0.04	2.45±0.08b
							ns		ns	
Fertiliser										
Control	21.35±3.6d	25.43±3.2d	9.69±0.9d	0.16±0.02c	0.17±0.02b	0.15±0.02b	0.20±0.00c	0.19±0.01c	1.43±0.03	2.54a±0.06b
ACD	40.97±2.7c	44.02±5.8c	43.65±5.0c	0.16±0.02c	0.16±0.02b	0.17±0.03a	0.24±0.01b	0.24±0.02b	1.48±0.07	2.39±0.10b
APM	53.45±8.3b	67.38±8.6b	55.58±9.5b	0.17±0.02b	0.20±0.03a	0.15±0.02b	0.25±0.01b	0.32±0.01a	1.40±0.05	2.66±0.06a
APM&CD	79.25±1.0a	76.88±2.4a	78.07±3.0a	0.20±0.02a	0.20±0.02a	0.17±0.02a	0.29±0.01a	0.29±0.02a	1.48±0.05	2.67±0.06a
									ns	
S*T	*	*	*	*	*	*	*	*	ns	ns

Note. Means with the same letter (s) in a column are not significantly different at the 5% level of probability by Duncan Multiple Range Test (DMRT); ns: not significant

Table 7
Influence of land-use type and soil amendment interactions on AMF spore abundance, soil available phosphorus, total nitrogen, and plant phosphorus uptake

Soil	Fertiliser	Total spore/50g soil dwtwk3	Total spore/50g soil dwtwk6	AVAILABLE Pwk3	AVAILABLE Pwk6	AVAILABLE Pwk9	S%N WK3	S%N WK6	S%N WK9	plt%P wk6	plt%P wk9
ASAR	Control	79.67±3.2c d	159.33±4.7d	0.25±0.017c	0.24±0.004d	0.23±0.003c	0.21±0.004ef	0.19ef±0.006	0.25±0.017c	0.24±0.004d	0.23±0.003c
	ACD	134.33±4.9 a	268.67±4.8a	0.24±0.004c	0.25±0.001c	0.27±0.002a	0.22d±0.011f	0.28±0.011bed	0.24±0.004c	0.25±0.001c	0.27±0.002a
	APM	122.33±6.5ab	244.67±5.0ab	0.26±0.001b	0.32±0.004a	0.25±0.002b	0.22±0.021ef	0.33±0.011ab	0.26±0.001b	0.32±0.004a	0.25±0.002b
	APM&CD	83.33±2.0d	166.670±2.2d	0.31±0.006a	0.28±0.004b	0.26±0.003b	0.27±0.018ab	0.34±0.023a	0.31±0.006a	0.28±0.004b	0.26±0.003b
BFR	Control	127.67±8.0a	255.33±3.8a	0.11±0.001g	0.13±0.002g	0.11±0.002f	0.20±0.005ef	0.20±0.006ef	0.11±0.001g	0.13±0.002g	0.11±0.002f
	ACD	96.67±6.2cd	193.33±3.8cd	0.12±0.001fg	0.11±0.002i	0.11±0.001f	0.23d±0.009ef	0.18±0.013g	0.12±0.001fg	0.11±0.002i	0.11±0.001f
	APM	137.00±6.7a	274.00±1.5a	0.12±0.004fg	0.13±0.002g	0.11±0.000f	0.29±0.002a	0.32±0.014ab	0.12±0.004fg	0.13±0.002g	0.11±0.000f
	APM&CD	126.33±9.4a	252.67±9.4a	0.14±0.001de	0.16±0.001e	0.12±0.003e	0.28±0.006a	0.30±0.006abc	0.14±0.001de	0.16±0.001e	0.12±0.003e
CRR	Control	92.00±19.7cd	184.00±4.9cd	0.13±0.001efg	0.12±0.003h	0.11±0.008f	0.20±0.006f	0.18±0.034g	0.13±0.001efg	0.12±0.003h	0.11±0.008f
	ACD	123.00±13.9ab	246.00±3.9ab	0.13±0.001def	0.14±0.000fg	0.13±0.003e	0.26±0.001abc	0.25±0.032cde	0.13±0.001def	0.14±0.000fg	0.13±0.003e
	APM	105.33±9.7bc	210.67±7.8bc	0.14±0.001de	0.14±0.002f	0.10±0.002g	0.24±0.005bcd	0.32±0.035ab	0.14±0.001de	0.14±0.002f	0.10±0.002g
	APM&CD	94.67±1.7cd	189.33±2.0cd	0.1500.001±d	0.16±0.001e	0.14±0.002d	0.30±0.029a	0.23±0.006def	0.1500.001±d	0.16±0.001e	0.14±0.002d

Note. Means with the same letter (s) in a column are not significantly different at the 5% level of probability by Duncan Multiple Range Test (DMRT); ns: not significant

fallow land-use type (BFR) with APM interaction also showed notably high sporulation at 137.00 and 274.00 spores at Weeks 3 and 6.

Available phosphorus levels in the ASAR soil-PM-CD interaction were significantly higher, measuring 81.86 mg/kg and 92.25 mg/kg at Weeks 3 and 6, respectively, compared to control treatments. The BFR soil-PM-CD interaction maintained consistently high phosphorus levels throughout the sampling period, ranging from 79.82 to 80.24 mg/kg.

The soil from cassava land-use type (CRR) showed moderate phosphorus values between 76.08 and 80.23 mg/kg. Soil nitrogen content was significantly higher in the ASAR soil x PM and ASAR soil x PM-CD interactions at 0.32% and 0.31%, respectively. Increased nitrogen levels were also observed in BFR and CRR soils. Plant phosphorus absorption corresponds with these trends, with values of 0.34% in ASAR soil x PM-CD and 0.32% in BFR soil x PM. The ASAR soil x CD interaction recorded the highest AMF spore counts, supporting cow dung's main ability to facilitate the sporulation process, which had been reported by previous studies on organic amendment influence (Siegenthaler et al., 2022). ASAR soil x PM was also effective, showing poultry manure's capability to support AMF development. The ASAR soil x PM-CD interaction recorded the highest phosphorus concentrations, revealing the combined benefits of organic inputs on nutrient mobilisation (Wu et al., 2024). Meanwhile, the BFR soil x PM-CD interaction maintained phosphorus concentrations, likely reducing cassava-induced allelopathic effects and making nutrient availability possible (Adamczyk et al., 2021). Increased nitrogen in ASAR soil x PM and ASAR soil x PM-CD also confirms the role of these combinations in improving nitrogen mineralisation and microbial turnover (Han et al., 2022). These findings affirm the effectiveness of soils from land-use types-organic amendment interactions in nutrient transfer and soil fertility enhancement (Lu et al., 2023).

Influence of Different Land-use Type on Plant Height and Number of Leaves

Plant height (PLTHt) and number of leaves (NOofLVS) were determined in three land-use types of soils, namely African star apple (ASAR), bush fallow (BFR) and cassava (CRR) at Weeks 3, 6 and 9 (Table 8). Plant height was not significantly different among land-use types in Week 3. However, plants in ASAR and BFR soil were much taller than those in CRR soil at Week 6. By Week 9, the plants supported by ASAR soil were the tallest, whereas BFR and CRR soils were comparatively less in stature.

For leaf production, ASAR and CRR soils recorded significantly higher numbers of leaves than BFR soil in Week 3. By Week 9, the application of poultry manure and cow dung (PM & CD) resulted in the significantly highest number of leaves when compared with other treatments. Although plant height was not significantly influenced by soil amendments at any growth stage, the PM & CD application led to a notable increase in leaf number by Week 9.

Table 8
Influence of different land-use types on plant height, number of leaves, shoot dry weight and pod weight

Treatment	PLTHt WK3	PLTHt WK6	PLTHt WK9	NO of LVS WK3	NO of LVS WK6	NOofLVS WK9	DRYSHTBM WK6	DRYSHTBM WK9	PODWT WK9
Soil									
ASAR	42.96±1.5	100.92±.4.6a	95.17±3.7a	11.00±0.0a	18.83±1.6	24.42±1.0	2.13±0.2	2.45±0.1	1.55±0.4
BFR	40.71±2.3	95.40±4.6a	73.67±7.2b	10.00±0.4b	15.92±1.4	26.33±1.8	1.58±0.3	2.33±0.4	1.64±0.7
CRR	41.04±1.0	77.04±4.8b	62.83±6.0b	11.42±0.3a	17.17±0.8	23.17±1.0	1.52±0.2	1.79±0.2	0.61±0.2
	ns	*	*	*	ns	ns	ns	ns	ns
Soil Amendment									
Control	38.89±0.8	93.89±2.4	69.00±6.6	10.33±0.4	14.67±1.4	22.22±1.0b	1.63±0.3	1.97±0.2	1.27±0.3
ACD	43.06±2.9	84.94±6.9	78.33±8.2	11.22±0.6	17.44±1.0	24.78±a1.3b	1.78±0.3	2.21±0.3	1.79±0.8
APM	44.39±1.8	89.64±7.0	71.89±9.9	10.67±0.3	17.00±1.2	23.67±1.6b	1.76±0.3	2.45±0.4	1.19±0.4
APM&CD	39.94±1.3	96.00±7.8	89.67±6.0	11.00±0.0	20.11±2.0	27.89±1.8a	1.79±0.3	2.12±0.2	0.83±0.5
	ns	ns	ns	ns	ns		ns	ns	ns
S*T	ns	ns	ns	ns	ns	*	ns	ns	ns

Note. Means with the same letter (s) in a column are not significantly different at the 5% level of probability by Duncan Multiple Range Test (DMRT); ns: not significant

There were no significant differences in dry shoot biomass or pod weight across the land-use type soils or amendment treatments by the end of the study period. Vegetative performance followed similar trends. Significantly taller plants in ASAR and BFR soils at Week 6 point to improved nutrient and microbial conditions in these soils (Alguacil et al., 2017; Aytenew & Bore, 2020). ASAR soil maintained a consistent increase in plant height up to Week 9, suggesting superior soil support for growth, compared to the reduced height observed in BFR and CRR soils, which may reflect nutrient depletion or biotic stress (Qu et al., 2020; Volpiano et al., 2022). Higher leaf numbers in ASAR and CRR soils at Week 3 indicate early soil advantages (Ofori et al., 2021). By Week 9, PM & CD treatment application resulted in the highest leaf production, demonstrating synergistic nutrient absorption impacts (Medina & Azcón, 2010; Pu et al., 2023). Although amendment type did not significantly affect plant height, the vegetative benefits of PM & CD treatment were apparent following their application. However, no significant gains in dry shoot biomass or pod weight were observed; this suggests there is a need for long-term investigation to evaluate yield impacts (Larkin, 2020). These outcomes point out the cruciality of integrated nutrient strategies to optimise crop productivity (Pantelides et al., 2023).

Correlation Between Total Spore Count, Available Phosphorus, and Plant Phosphorus (%P) and Nitrogen (%N) Content Over Time (Weeks 3, 6, and 9)

At Week 3, a perfect correlation (r = 1.000**) was observed for AMF spore counts, confirming a consistent pattern in AMF abundance (Table 9). A strong positive correlation was also recorded between available phosphorus at Week 3 and 6 (r = 0.857**, $p < 0.01$), indicating that early higher phosphorus concentration in the experiment was maintained over time. Similarly, available phosphorus at Week 3 significantly correlated with values at Week 9 (r = 0.752**, $p < 0.01$), confirming a sustained phosphorus availability pattern. Available phosphorus at Week 6 showed a significant positive correlation with plant phosphorus uptake (r = 0.585**, p < 0.01), indicating its important role in supporting plant development. A moderate but significant correlation was also observed between available phosphorus at Week 3 and plant nitrogen at Week 6 (r = 0.353*, p < 0.05), suggesting that early phosphorus availability was associated with subsequent nitrogen assimilation.

Finally, strong correlations between available phosphorus at Weeks 3 and 6 and plant uptake support biological processes in the soil and phosphorus availability (Johnston & Poulton, 2019; Pang et al., 2024). The link between Week 6 phosphorus and plant uptake stresses the importance of nutrient timing in crop production (Khan et al., 2023). The observed association between phosphorus and nitrogen unveils their interactive influence in plant nutrition (Zhao et al., 2021), consistent with earlier reports of AMF-driven coordination of N and P uptake (Krouk & Kiba, 2020; Schleuss et al., 2020). These findings collectively demonstrate that land-use types- organic amendment interactions significantly

Table 9
Relationships between AMF spore abundance, soil available phosphorus, plant phosphorus (%) and nitrogen (%) content across sampling periods

	Total spore/50g soil dwtwk3	**Total spore/50g soil dwtwk6**	**Total spore/50g soil dwtwk9**	**AVAILABLE Pwk3**	**AVAILABLE Pwk6**	**AVAILABLE Pwk9**	**plt%P wk6**	**plt%P wk9**	**plt%N wk6**
Total spore/50g soil dwtwk3	1.000**	-	-	-	-	-	-	-	-
Total spore/50g soil dwtwk6	.248	.248	-	-	-	-	-	-	-
Total spore/50g soil dwtwk9	.163	.163	.181	-	-	-	-	-	-
AVAILABLE Pwk3	.212	.212	.278	.904**	-	-	-	-	-
AVAILABLE Pwk6	.141	.141	.405*	.849**	.869**	-	-	-	-
AVAILABLE Pwk9	.131	.131	.121	.752**	.651**	.608**	-	-	-
plt%P wk6	.322	.322	-.120	.569**	.585**	.464**	.379*	-	-
plt%P wk9	.014	.014	.090	.113	.124	.045	-.024	.008	-
plt%N wk6	.061	.061	-.201	.353*	.396*	.187	.070	.581**	.237

facilitate nutrient cycling and retention as well as plant nutrient uptake efficiency within a biodiverse ecosystem.

CONCLUSION

This study demonstrates that soils from different land-use types and organic amendments significantly influenced arbuscular mycorrhizal fungi (AMF) activity in the cowpea rhizosphere, as reflected in spore abundance, root colonisation, nutrient availability, and plant nutrient uptake. Soil from the cassava land-use type (CRR) promoted greater AMF root colonisation and favoured *Gigaspora* spp., whereas African star apple soil (ASAR) supported higher *Glomus* abundance and consistently improved soil nitrogen, plant phosphorus and nitrogen uptake, as well as vegetative growth. Cow dung enhanced AMF sporulation, while poultry manure promoted root colonisation, indicating that the organic amendments influenced different aspects of AMF development. The combined application of poultry manure and cow dung further increased soil phosphorus availability and leaf production, highlighting the benefits of integrated organic nutrient management. Although dry shoot biomass and pod weight were not significantly affected during the study period, the improvements in AMF activity, nutrient uptake, and vegetative growth suggest that combining appropriate land-use practices with organic amendments can improve the soil biological environment and has the potential to support better cowpea productivity under longer-term or field conditions. Overall, these findings emphasise the value of integrating suitable land-use systems with organic nutrient management to enhance AMF-mediated soil fertility and promote sustainable cowpea production.

IMPLICATIONS OF THE STUDY

The findings indicate that careful selection of organic amendments, combined with inherent soil properties, can enhance AMF activity, improve nutrient uptake, and support sustainable soil fertility management. Specifically, the application of cow dung in soils from African Star Apple (ASAR) and bush fallow (BFR) land-use types, and poultry manure in soil from cassava land-use type (CRR), emphasises the importance of site-specific amendment strategies for maximising AMF benefits in tropical agroecosystems.

LIMITATIONS OF THE STUDY AND RECOMMENDATIONS FOR FUTURE RESEARCH

AMF identification was based on spore morphology, which may not fully capture community diversity compared with molecular-based approaches. The experiment was conducted under specific site conditions, and responses may differ across other agroecological zones or soil parent materials. Future studies should incorporate molecular techniques (e.g., high-throughput sequencing) to provide a more detailed assessment of

AMF community composition and functional diversity. Additionally, crop yield, plant physiological responses, and AMF-mediated stress tolerance should be evaluated to further strengthen the agronomic relevance and application of AMF-based soil fertility strategies.

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The Effect of Cooking Time on Physical, Cooking, and Textural Properties of Parboiled Rice MR297

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ABSTRACT

Parboiled rice is widely consumed across Asia for its enhanced nutritional retention and distinct texture, and cooking duration strongly affects its final eating quality. Cooking-time effects have mostly been studied for established varieties, however, and remain unexamined for the recently released Malaysian cultivar MR297. This study evaluated the effects of cooking time on the physical, cooking, and textural properties of parboiled MR297, benchmarked against unparboiled MR297 and commercially parboiled Malaysian rice. Samples were cooked for 4, 8, 12, 16, 20, and 24 minutes and analysed for physical dimensions, colour (L*, a*, b*), cooking properties (elongation ratio, moisture sorption, l/b ratio), and textural attributes (hardness, cohesiveness, chewiness, adhesiveness, springiness). Data were analysed by one-way ANOVA with Tukey's test ($p<0.05$), followed by principal component analysis (PCA). Cooking duration significantly affected textural, physical, colour, and cooking properties of all samples ($p<0.05$). Hardness in parboiled MR297 decreased markedly from 13.26 to 2.55 N, with parallel reductions in cohesiveness and chewiness, while springiness remained unchanged ($p>0.05$). L* rose from 55.43-57.80 to 64.47-65.02, elongation ratio rose to 1.34-1.46, and moisture sorption increased three- to four-fold ($p<0.05$); l/b ratio was unaffected ($p>0.05$). Textural properties largely plateaued by 16 min, whereas moisture sorption kept rising beyond this point, indicating ~16 min offers the best balance between softness and over-hydration for MR297. PCA showed PC1 distinguished samples by cooking duration, and PC2 separated unparboiled from parboiled rice. Overall, cooking time critically determines

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parboiled MR297 quality, with prolonged cooking yielding softer, more elongated, offering processors evidence-based cooking guidance for this cultivar.

Keywords: Cooking, cooking properties, MR297 rice, parboiling, physical properties, textural properties

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for almost half of the global population. The International Rice Genebank at the International Rice Research Institute (IRRI) houses approximately 117,000 distinct rice varieties, encompassing both cultivated and wild types (Dutta & Mahanta, 2014). MARDI released MR297 in 2014. This Malaysian rice cultivar was bred locally to enhance disease resistance compared to the MR263 cultivar (Azmi, 2016). Still, the cooking and eating-quality traits are insufficiently characterised. This covers the behaviour under various cooking conditions. As a result, there are still high uncertainties about whether millers and consumers will accept it.

Nevertheless, the milling yield stands at 61.4%, and the head rice yield is just 67.8%, both of which fall below the corresponding values of MR 263, which are 65.5% and 88%, respectively. The economic value of the variety to milling factories may be negatively impacted as a consequence. Parboiling is the hydrothermal procedure used to pretreat raw paddy. To produce parboiled rice, the paddy (or brown rice) must undergo a process of soaking, cooking, and drying before it can be subjected to milling (Qin & Siebenmorgen, 2005). To obtain the highest possible amount of head rice and minimise breakage, which is a significant loss during processing, it is recommended to first parboil the paddy to gelatinise the starch (Buggenhout et al., 2014; Muchlisyiyah et al., 2024a). Parboiling is generally defined as mild steaming of rice, which alters its physical and chemical components, thus promoting digestibility, enhancing texture, increasing milling yield as well as lowering amylose loss during cooking (Dutta & Mahanta, 2012; Iqbal et al., 2021; Roy et al., 2019). Parboiling thus represents a feasible means to mitigate the milling liabilities of MR297, if it is able to balance quality and willingness to eat.

The modification of characteristics during parboiling would likewise result in distinct cooking properties. The texture of cooked rice is a vital element in assessing its quality and is frequently utilised as a gauge of consumer satisfaction and choice. The cooking process is associated with the capacity of water to moisturise the inner part of a rice grain while cooking (Karizaki, 2016). Upon cooking, rice starch absorbs water, hydrates and gelatinises while releasing amylose. The cooking time and temperature also largely influence the resulting physicochemical and textural properties (Chakkaravarthi et al., 2008; Meresa et al., 2020; Shinde et al., 2014). Processing or parboiling duration effects on texture and other physicochemical properties have been previously studied for different rice varieties, but not for black Sri Lankan rice. For example, Onmankhong et al. (2021) found that hardness and

toughness were greatly affected by processing parameters in parboiled Thai rice. Similarly, for several germinated rice varieties, parboiling duration induced systematic changes in cooking, textural and pasting properties (Mudgal & Singh 2024). Although none of these studies investigated MR297. Nor is there any study that has compared parboiled samples of their respective paired raw and commercial parboiled reference for the full range of domestic cooking times utilised in food preparation. After all, this is the sort of comparison required in order to provide real-world, variety-specific guides to cooking.

In Malaysia, a wide range of imported rice types are available in the market, including jasmine, basmati, normal white, waxy, parboiled, and brown rice. These rice varieties from other countries (e.g., Thailand, Vietnam, India, Pakistan and the USA (Jamal et al., 2013), that any competitive local variety must be evaluated with respect to cooking and textural performance. The cooking-time behaviour of parboiled MR297 is also unknown at this time, as mentioned above. Since the effect of parboiling on starch structure is dependent on both variety and processing conditions (Dutta & Mahanta, 2012), one cannot simply assume that findings from other cultivars would extrapolate to these also. We postulate that the cooking time imparts systematic, measurable changes in the textural, physical, colour, and cooking attributes of parboiled mineral-rich 297 — changes which differentiate it from its raw and commercial equivalents. This profile serves two purposes. This provides millers and researchers with their first cooking-time reference for MR297. Second, it alleviates the problems regarding whether parboiling can offset the lower milling yield of this poorest quality rice cultivar with benefits for cooking. This work is novel in comparing MR297 over a full cooking-time range to an unparboiled sample and, at the same time, with a commercial parboiled benchmark. There has been no previous report comparing the three parameters. Consequently, this study examined the influence of cooking time (4-24 min) on certain physical properties (dimension and colour), cooking properties (elongation ratio, moisture sorption, l/b ratio) as well as textural properties (hardness, adhesiveness, cohesiveness, springiness and chewiness) in parboiled rice MR297 compared to both raw MR297 and its commercially processed parallel.

MATERIALS AND METHODS

Materials

A local farmer in Tanjung Karang, Selangor, Malaysia provided fresh paddy (MR297). The researchers randomly picked fresh paddy grain and transported it to the laboratory. The grain was then stored in a tightly sealed container at 10°C in a cold room until it was ready for further processing (Reddy & Chakraverty, 2004). The initial moisture content was 19.5%. Other plant parts and foreign materials were removed manually; the fresh paddy sample MR297 was sun-dried for two consecutive days until the moisture content (MC) decreased to 12.1%.

"Jati Rebus" brand parboiled rice was obtained from a local store in Malaysia and used as the commercially parboiled rice for comparison purposes.

Parboiling Process

The parboiled MR297 was prepared by subjecting fresh MR297 paddy to a hot soaking method in a laboratory. A 100 mL amount of water was heated to 54.8 °C in a 250 mL beaker using a water bath (Memmert, WNB22, Germany) (Muchlisyiyah et al., 2025). Aluminium foil was used to completely seal the container in order to prevent excessive evaporation. Paddy (100 g) was added to the water in the glass beaker. The ratio of rice to water was 1:1 (Jannasch & Wang, 2020). A modest agitation was performed to guarantee thorough immersion of the paddy. The paddy was immersed for a period of 2 hours. The rice was then steamed for 20 minutes at a temperature of 100 °C using a domestic steamer (TEFAL VC1401, FRANCE). The rice was cooled at ambient temperature for a period of one hour (Bootkote et al., 2016). The paddy was then subjected to dehydration in an oven (Memmert DO6836, Germany) at 38 °C for 24 hours (Roy et al., 2019; Tian et al., 2018; Villanova et al., 2017). The hull removal process from dry parboiled rice was conducted with a Satake THU-35 dehusker (THU-35, Satake Corp., Japan), followed by a polishing time of 2.5 minutes using a TM05-C mill (Satake, Japan) (Liang et al., 2008).

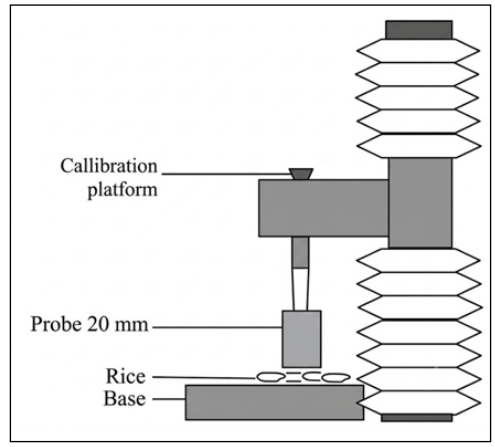
Cooking Process

Three kernel types (unparboiled MR297, parboiled MR297, and commercial local Malaysian parboiled rice) were cooked in water (rice: water = 1:2 for unparboiled rice; rice: water = 1:2.5 for parboiled rice) at 100°C at selected cooking times (4, 8, 12, 16, 20, and 24 min) (Syafutri et al., 2016). Cooking times were chosen to cover the range of under- and fully-cooked, as well as slightly overcooked kernels, also covering the full softening-moisture uptake trajectory. A higher rice-to-water ratio was adopted for parboiled-plated (1:2.5) than for un-parboiled plated rice at the rate of 1:2. That is, the starch structure that results from parboiling in a gelatinised and hydrated yet more compact form needs a relatively larger amount of free water to achieve equivalent hydration/cooking in the same time and space. This bulk-cook method was applied to analyses of thickness, size and hue described below. For the elongation-ratio and moisture-sorption measurements, a different protocol (also detailed below) was used on a smaller scale here using assay conditions established from literature.

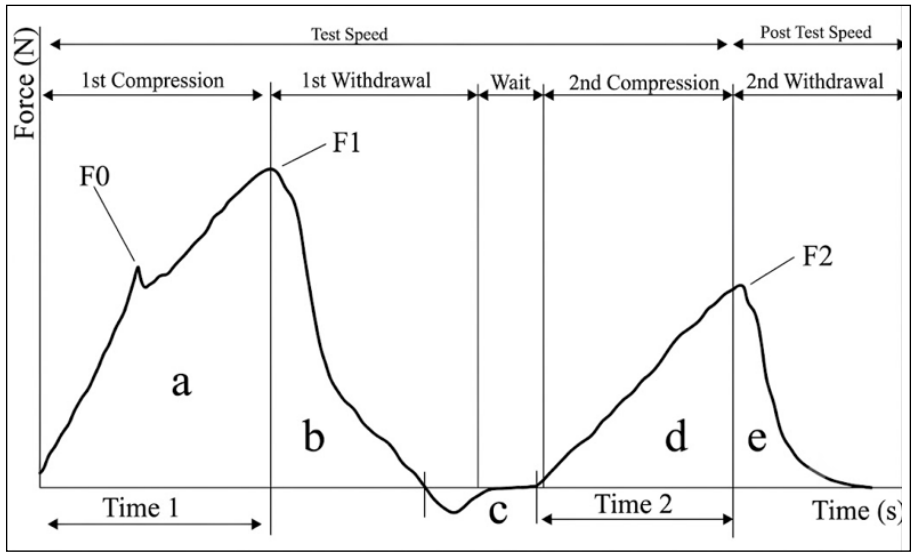
Textural Properties Determination

The texture profile analysis (TPA) was conducted promptly upon the cooked rice samples attaining ambient temperature, using the modified technique outlined by Chusak et al. (2019). Thirty kernels from each cooked rice sample were selected and placed at the bottom

of the device. A TA.XT-Plus Texture Analyser, equipped with a 20 mm cylindrical probe attachment from Technologies Corp. and Stable Micro Systems Ltd., MA, was employed using a standard two-cycle compression method. The schematic diagram for the textural analysis can be seen in Figure 1a. The probe was permitted to fall at a rate of 1 mm/s, then ascend and then repeat the compression cycle. The compression was adjusted to a strain of 75%. Textural characteristics include hardness, adhesiveness, cohesion, springiness, gumminess, and chewiness.



1a.



1b.

Figure. 1a. Schematic diagram for rice textural analysis (Adapted and modified from Liu et al., 2019). 1b. Texture Profile Analysis (TPA) curve resulting from the testing of rice. The graph displays the Force (N) versus Time (s) profile from two compression cycles. Peak F1 represents the hardness of the first cycle, F2 represents the hardness of the second cycle, and Area 3 (the negative area) indicates adhesiveness

The hardness (N) corresponds to the maximum force observed during the initial compression. Adhesiveness (N · s) refers to the integral of the curve for the initial negative peak. Cohesiveness is calculated by dividing the area under the second compression curve by the area under the first compression curve. Springiness, expressed as a percentage, represents the ratio of a product's ability to regain its previous height after compression. Chewiness is defined by multiplying the values of Hardness, Cohesiveness, and Springiness.

The analysis for the textural parameters can be seen in Equations 1-5 and illustrated in Figure 1b:

$$Hardness = F1 \quad [1]$$

$$Adhesiveness = c \quad [2]$$

$$cohesiveness = \frac{(d+e)}{(a+b)} \quad [3]$$

$$springiness\ (\%) = \frac{Distance\ \ 2}{distance\ \ 1} \times 100 = \frac{Time\ \ 2}{Time\ \ 1} \times 100 \quad [4]$$

$$Chewiness\ (\%) = \frac{F1xDistance\ \ 2}{distance\ \ 1 \times \frac{(d+e)}{(a+b)}} \quad [5]$$

Colour Properties Determination

Milled rice grains were randomly picked and placed in a Petri dish. The colour reader (FRU WR10, China) was calibrated using a white and black whiteboard. L* (lightness), a* (green-red), and b* (blue-yellow) scale of the rice types were determined by placing the colour meter adjacent to the petri dish containing the samples and photographing it using a 40 mm diameter lens with a white blank paper background (Kumar et al., 2022).

Dimensional Properties

A digital vernier calliper with a precision of 0.01 mm was utilised to measure the dimensions of 100 randomly selected rice grain heads, namely their length (L) and width (W). The measured dimensional properties are displayed in Figure 2.

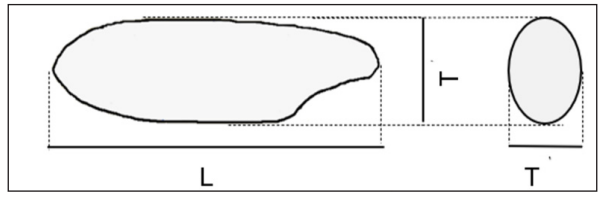


Figure 2. Dimensional characteristics of MR297 rice grain: length, L, width, W; thickness, T
Source: Muchlisyiyah et al. (2024b)

Cooking Properties Determination

The assessment of cooking properties was performed according to Ojediran et al. (2020) and Singh et al. (2005). The parameters measured were the l/b (length-to-breadth) ratio, moisture sorption, and elongation ratio. Head rice (25 grains) samples were randomly selected from milled rice grains and measured for length (L) and width (W) using a digital vernier calliper. The l/b ratio was then computed by dividing the total length by the total width (Equation 6).

$$L/_b\, ratio = \frac{total\ length\ of\ 25\ rice\ kernels}{total\ width\ of\ 25\ rice\ kernels} \quad [6]$$

5 g of head rice samples from each cultivar were cooked in 10 ml of distilled water in a boiling water bath. The rice was removed after 4, 8, 12, 16, 20, and 24 min. The surface water on the cooked rice was absorbed by pressing the cooked samples on filter paper sheets. Next, the cooked samples were precisely weighed, and the moisture sorption was determined (Equation 7).

$$Moisture\ sorption = \frac{weight\ of\ \ cooked\ rice - weight\ of\ \ raw\ rice}{weight\ of\ raw\ rice} \quad [7]$$

The cumulative length of 10 cooked rice kernels was divided by the length of 10 uncooked raw kernels to calculate the elongation ratio (Equation 8).

$$Elongation\ Ratio\ (ER\%) = \frac{Length\ of\ 25\ \ cooked\ rice\ kernels}{Length\ of\ 25\ \ raw\ rice\ kernels} \quad [8]$$

Statistical Analysis

Each experiment was performed in triplicate. Minitab (Version 19, LLC, USA) was used to conduct all statistical analyses, representing three independent parboiling/cooking batches processed and analysed separately. The results are expressed as the average standard deviation. The significance level for analysis of variance (ANOVA) differences was set at $p<0.05$. The principal component analysis (PCA) was also carried out using the Minitab Software (Version 19, LLC, USA).

RESULTS AND DISCUSSIONS

Textural Properties

Figure 3 shows the cooked rice grains at each cooking time interval, and Table 1 gives the corresponding hardness values. Hardness decreased with cooking time in all three samples. The decline was steeper at first and became more gradual thereafter. A similar pattern has

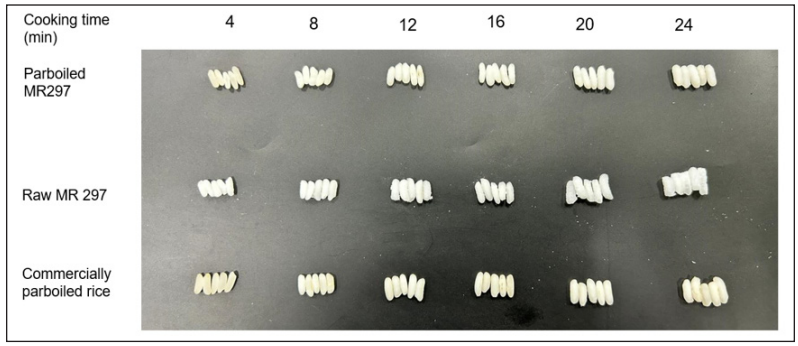


Figure 3. Cooked rice grains in different cooking times

been reported for other starchy foods during cooking (Beleia et al., 2004; Chusak et al., 2019; Gavahian et al., 2019; Saleh & Meullenet, 2013). This biphasic trend reflects the advance of the gelatinisation front through the kernel. Water and heat reach the outer layers first, disrupting the crystalline structure there and softening the surface relatively quickly. The denser core hydrates more slowly, so gelatinisation is completed there only later in cooking. Softening slows once the outer starch has gelatinised, having lost crystallinity, absorbed water, and swollen with continued amylose release (Li et al., 2016). The result is a more hydrated and looser matrix that resists compression less, which registers as the falling hardness values.

Adhesiveness is the measure of the labour or force required to overcome the attraction forces between a product's surface and the surface of the substance it contacts. Adhesiveness is conventionally defined in the negative here. A larger negative value corresponds to greater stickiness, whereas a smaller (less negative) number corresponds to less stickiness, not to "less adhesiveness" in some absolute sense. After 24 min, cooking produced -0.01 N·s for parboiled MR297, -0.14 N·s for unparboiled MR297, and -0.03 N·s for commercially parboiled rice, placing the commercial sample between the two MR297 samples in stickiness (Table 1). The magnitude of tackiness decreased for parboiled MR297 from -0.06 to -0.01 N·s, approaching zero as cooking time increased, indicating that the grains became progressively less sticky. In contrast, unparboiled and commercially parboiled rice showed no significant variation in adhesive properties across cooking times ($p>0.05$). Parboiled MR297 and commercially parboiled rice were both significantly less sticky than unparboiled rice at all stages of cooking. This is compatible with the well-documented function of parboiling in reorganising starch into a compact matrix, which retains amylose within the granule rather than liberating it to the kernel surface (Islam et al., 2004), and ultimately limits the kernel's capacity to develop surface stickiness from freed amylopectin accumulating during cooking (Mohapatra & Bal, 2006). Unparboiled rice adhesiveness in the present study was similar to values reported for white rice cooked in an electric cooker (Chusak et al., 2019).

Table 1
The effects of cooking time on the textural properties of MR297 parboiled rice

Rice	CT	Hardness (N)	Adhesiveness (N.sec)	Cohesiveness (peak2/peak1)	Springiness	Chewiness (N)
Parboiled rice MR297	4	13.26 ± 1.66^{a}	-0.06 ± 0.01^{c}	$0.83 \pm 0.03^{a-c}$	1.00 ± 0.00^{a}	9.99 ± 1.34^{a}
	8	$10.90 \pm 1.11^{b-c}$	$-0.05 \pm 0.01^{b-c}$	$0.71 \pm 0.06^{b-d}$	1.00 ± 0.00^{a}	7.73 ± 1.15^{b}
	12	$4.57 \pm 0.88^{d-g}$	$-0.02 \pm 0.01^{a-b}$	$0.67 \pm 0.06^{b-d}$	1.00 ± 0.00^{a}	$2.40 \pm 0.09^{d-g}$
	16	$2.94 \pm 0.58^{e-g}$	-0.02 ± 0.00^{a}	$0.66 \pm 0.03^{b-d}$	1.00 ± 0.00^{a}	$2.00 \pm 0.27^{f-g}$
	20	$2.68 \pm 0.18^{f-g}$	-0.02 ± 0.00^{a}	$0.63 \pm 0.02^{c-d}$	1.00 ± 0.00^{a}	$1.15 \pm 0.38^{e-g}$
	24	2.55 ± 0.66^{g}	-0.01 ± 0.00^{a}	0.62 ± 0.04^{d}	1.00 ± 0.00^{a}	1.11 ± 0.25^{g}
Unparboiled Rice	4	9.65 ± 1.46^{c}	-0.15 ± 0.01^{d}	0.92 ± 0.02^{a}	1.00 ± 0.00^{a}	$8.60 \pm 0.79^{a-b}$
	8	$5.25 \pm 0.32^{d-e}$	-0.15 ± 0.00^{d}	$0.81 \pm 0.07^{a-d}$	1.00 ± 0.00^{a}	$3.80 \pm 0.52^{d-e}$
	12	$4.71 \pm 0.98^{d-g}$	-0.15 ± 0.02^{d}	$0.80 \pm 0.06^{a-d}$	1.00 ± 0.00^{a}	$3.56 \pm 0.90^{d-e}$
	16	$4.04 \pm 0.28^{d-g}$	-0.14 ± 0.01^{d}	$0.78 \pm 0.02^{a-d}$	1.00 ± 0.00^{a}	$3.42 \pm 0.17^{d-e}$
	20	$4.36 \pm 0.18^{d-g}$	-0.15 ± 0.01^{d}	$0.77 \pm 0.10^{a-d}$	1.00 ± 0.00^{a}	$3.25 \pm 0.02^{d-e}$
	24	$3.94 \pm 0.32^{d-g}$	-0.14 ± 0.02^{d}	$0.74 \pm 0.06^{a-d}$	1.00 ± 0.00^{a}	$3.01 \pm 0.52^{d-f}$
Commercially parboiled Malaysian rice	4	$12.26 \pm 0.68^{a-b}$	-0.03 ± 0.00^{ab}	$0.85 \pm 0.07^{a-b}$	1.00 ± 0.00^{a}	$9.53 \pm 0.43^{a-b}$
	8	8.96 ± 0.47^{c}	-0.03 ± 0.01^{ab}	$0.85 \pm 0.07^{a-b}$	1.00 ± 0.00^{a}	7.67 ± 0.25^{b}
	12	5.59 ± 0.13^{d}	-0.03 ± 0.01^{ab}	$0.84 \pm 0.07^{a-b}$	1.00 ± 0.00^{a}	5.41 ± 0.23^{c}
	16	$5.00 \pm 0.64^{d-f}$	-0.03 ± 0.00^{ab}	$0.80 \pm 0.11^{a-d}$	1.00 ± 0.00^{a}	$3.71 \pm 0.13^{c-d}$
	20	$3.83 \pm 0.39^{d-g}$	-0.03 ± 0.00^{ab}	$0.79 \pm 0.12^{a-d}$	1.00 ± 0.00^{a}	$2.72 \pm 0.16^{d-g}$
	24	3.17 ± 0.45^{g}	-0.03 ± 0.00^{ab}	$0.74 \pm 0.04^{a-c}$	1.00 ± 0.00^{a}	$2.36 \pm 0.30^{d-g}$

Note. CT = cooking time (min). Data are expressed as mean ±SD; Different letters indicate statistically significant differences exist $p < 0.05$ for each column. Means that do not share a letter are significantly different. Tukey's test was applied with 95% simultaneous confidence intervals

All samples showed a gradual decrease in cohesiveness with increasing cooking time. Parboiled MR297 fell from 0.83 to 0.62, the unparboiled sample from 0.92 to 0.74, and commercial rice from 0.85 to 0.74. Cohesiveness was lower in parboiled than in unparboiled rice, and no significant difference was observed between unparboiled and commercial rice ($p>0.05$; Table 1). This decline shows that structure was reduced in a stepwise fashion over the course of cooking. As water diffuses in, starch gelatinises, and amylose leaches out, compromising the dense granule and protein network formed during parboiling (Islam et al., 2004) and decreasing structural recovery. These values are consistent with the 0.6 to 0.7 range reported by Chusak et al. (2019) and exceed the 0.55 reported by Bonto et al. (2018).

Chewiness is defined as the energy needed to chew a semi-solid food until it can be swallowed (Li et al., 2023). As shown in Table 1, it ranged from 9.99 to 1.11 N for parboiled MR297, from 8.60 to 3.01 N for unparboiled MR297, and from 9.53 to 2.36 N for commercially parboiled rice, with no statistically significant difference between samples after 20 min of cooking. As previously noted, the reduction in hardness and cohesiveness contributes to the decline in chewiness, since chewiness is a function of both acting together.

This decrease therefore reflects a general loosening of the grain structure as the kernel softens. Gumminess was calculated as an intermediate step and is not reported separately.

Springiness was evaluated as the ratio of recovery height after the second compression to that after the first (Tao et al., 2020). A value of 1.00 ± 0.00 was recorded in all samples at every cooking time and treatment ($p>0.05$; Table 1). This uniformity likely reflects a real characteristic of cooked rice, namely near-complete elastic height recovery under the 75% strain regime applied here, consistent with springiness values reported elsewhere for cooked rice as being close to 1.0. Because springiness did not change significantly between treatments, it was removed from the PCA (see Section Principal Component Analysis).

Dimensional Properties

During cooking, rice grains grow in all three dimensions (Febina et al., 2023; Table 2). Water moves progressively into the starchy endosperm, and as the starch granules take it up and swell, they push outward against the cell wall and protein matrix surrounding them; because that matrix is only semi-rigid, it yields, and the kernel stretches along its length,

Table 2
The effects of cooking time on the dimensional properties of MR297 parboiled rice

Rice	CT	Length (mm)	Width (mm)	Thickness (mm)
Parboiled rice MR297	4	$8.10 \pm 0.43^{f-g}$	2.19 ± 0.15^{g}	$1.92 \pm 0.14^{g-h}$
	8	$8.69 \pm 0.52^{d-e}$	2.43 ± 0.12^{f}	$2.03 \pm 0.07^{e-g}$
	12	$9.04 \pm 0.54^{c-e}$	$2.44 \pm 0.16^{e-f}$	$2.08 \pm 0.12^{d-f}$
	16	9.53 ± 0.53^{b}	$2.48 \pm 0.13^{d-f}$	$2.15 \pm 0.11^{c-e}$
	20	9.53 ± 0.50^{b}	$2.53 \pm 0.16^{c-f}$	$2.15 \pm 0.11^{c-e}$
	24	10.12 ± 0.68^{a}	$2.69 \pm 0.15^{a-b}$	$2.35 \pm 0.14^{a-b}$
Unparboiled Rice	4	7.51 ± 0.44^{h}	2.20 ± 0.16^{g}	1.85 ± 0.13^{h}
	8	8.06 ± 0.52^{g}	$2.44 \pm 0.19^{e-f}$	$1.99 \pm 0.14^{f-g}$
	12	$8.95 \pm 0.57^{c-e}$	$2.60 \pm 0.15^{a-e}$	$2.17 \pm 0.24^{c-d}$
	16	$9.14 \pm 0.66^{b-d}$	$2.63 \pm 0.15^{a-d}$	$2.23 \pm 0.14^{b-c}$
	20	$9.22 \pm 0.59^{b-c}$	$2.64 \pm 0.19^{a-c}$	$2.31 \pm 0.17^{a-b}$
	24	$9.28 \pm 0.68^{b-c}$	$2.70 \pm 0.23^{a-b}$	2.41 ± 0.22^{a}
Commercially parboiled Malaysian rice	4	$8.10 \pm 0.30^{f-g}$	2.18 ± 0.12^{g}	$1.93 \pm 0.06^{g-h}$
	8	$8.57 \pm 0.27^{e-f}$	2.39 ± 0.17^{f}	$2.04 \pm 0.07^{d-g}$
	12	$9.03 \pm 0.31^{c-e}$	2.43 ± 0.12^{f}	$2.09 \pm 0.07^{d-f}$
	16	9.53 ± 0.25^{b}	$2.48 \pm 0.16^{d-f}$	$2.15 \pm 0.09^{c-e}$
	20	9.54 ± 0.27^{b}	$2.54 \pm 0.18^{b-f}$	$2.15 \pm 0.09^{c-e}$
	24	10.12 ± 0.35^{a}	2.71 ± 0.17^{a}	2.35 ± 0.11a-b

Note. CT = cooking time (min). Data are expressed as mean ±SD; Different letters indicate statistically significant differences exist $p < 0.05$ for each column. Means that do not share a letter are significantly different. Tukey's test was applied with 95% simultaneous confidence intervals

width, and thickness. Expansion measured at a given cooking time is therefore a proxy for how deeply the hydration front has penetrated. Across the 4-24 min range, grain length rose significantly ($p<0.05$) in all samples: 8.10 to 10.12 mm in parboiled MR297, 7.51 to 9.28 mm in unparboiled rice, and 8.10 to 10.12 mm in the commercial rice, the parboiled and commercial samples converging on the same final value.

Width showed the same trend. It rose from 2.19 to 2.69 mm in parboiled MR297, 2.20 to 2.70 mm in the unparboiled rice, and from 2.18 to 2.71 mm in the commercial sample ($p<0.05$). All three reached a comparable width by the end of the cooking range. Thickness increased in parallel: 1.92 to 2.35 mm in parboiled MR297, 1.85 to 2.41 mm in the unparboiled rice, and 1.93 to 2.35 mm in the commercial sample ($p<0.05$). Together, the three dimensions point to the same conclusion. Temperature was held at 100°C throughout, so heat input cannot explain the differences in expansion. Cumulative water uptake is therefore the governing factor, consistent with a hydration-driven swelling mechanism.

Colour Properties

Prior research has demonstrated that a decrease in the intensity of colour in cooked rice improves consumer satisfaction and increases its market worth (Tian et al., 2014). The rice's L* increased from 56.92 ± 2.04 to 64.74 ± 0.30 after being cooked for 24 minutes, which was comparable to the L* of the commercially parboiled rice samples. Nevertheless, the extended duration required to fully cook the unparboiled rice MR297 resulted in an elevation in the rice's L*, reaching 57.80 ± 0.0.10 and 65.02 ± 0.68 after 0 to 24 minutes of cooking time, respectively. Unparboiled MR297 rose from 57.80 ± 0.10 to 65.02 ± 0.68 over the same 4-24 min range ($p<0.05$). L* increased in all samples. As water penetrates the kernel, the crystalline structure of the starch granules breaks down, and the pigmented surface layers are diluted. The result is a lighter grain than the raw kernel.

Regarding the a* value, as depicted in Table 3, it can be noticed that the a* value of rice grain decreased with cooking time from -0.30 to -0.61, from -0.59 to -1.51, and from 1.11 to -1.39 ($p<0.05$) for parboiled rice MR297, unparboiled MR297, and commercially parboiled rice MR297, respectively. At a 24-minute cooking time, the a* value of commercially parboiled rice was not significantly different from that of parboiled rice MR297 and unparboiled rice MR297. Meanwhile, the a* value of unparboiled MR297 is significantly different from that of parboiled rice MR297 at the final cooking time. The shift toward green is most likely a dilution effect. Parboiling and husk removal leave a brownish pigmentation on the grain surface. As the kernel takes up water and lightens, that pigmentation is progressively masked, which fits the rise in L* described above. The pigment composition itself does not appear to change.

Table 3
The effects of cooking time on the colour properties of MR297 parboiled rice

Rice	CT	**L***	a*	b*
Parboiled rice MR297	4	56.92 ± 2.04g-h	-0.30 ± 0.19b	12.24 ± 0.06^{c}
	8	59.14 ± 1.07^{e-g}	-0.30 ± 0.12^{b}	10.17 ± 0.28^{d}
	12	59.97 ± 0.20^{d-g}	-0.45 ± 0.36^{b-d}	8.63 ± 0.26^{d-e}
	16	61.54 ± 0.81^{b-e}	-0.37 ± 0.08^{b-c}	7.63 ± 1.40^{e-f}
	20	63.01 ± 0.52^{a-d}	-0.45 ± 0.05^{b-d}	6.48 ± 1.27^{f-g}
	24	64.74 ± 0.30^{ab}	-0.61 ± 0.14^{b-e}	5.50 ± 0.52^{g}
Unparboiled Rice	4	57.80 ± 0.10^{f-h}	-0.59 ± 0.05^{b-e}	5.96 ± 0.64^{f-g}
	8	59.91 ± 0.40^{d-g}	-0.71 ± 0.01^{b-f}	3.23 ± 0.42h
	12	60.89 ± 0.17^{d-f}	-0.92 ± 0.05^{b-f}	2.32 ± 0.50^{h-i}
	16	61.15 ± 0.15^{c-e}	-1.17 ± 0.02^{c-f}	1.67 ± 0.10^{h-i}
	20	62.78 ± 2.90^{a-d}	-1.22 ± 0.02^{d-f}	1.26 ± 0.06^{h-i}
	24	65.02 ± 0.68^{a}	-1.51 ± 0.07^{f}	1.17 ± 0.47^{i}
Commercially parboiled Malaysian rice	4	55.43 ± 1.63^{h}	1.11 ± 0.88^{a}	19.16 ± 0.42^{a}
	8	56.87 ± 0.71^{g-h}	0.59 ± 0.42^{a}	16.87 ± 0.83^{b}
	12	60.59 ± 1.21^{d-f}	-0.79 ± 0.26^{b-f}	15.32 ± 0.85^{b}
	16	64.29 ± 0.09^{a-c}	-1.14 ± 0.04^{c-f}	10.43 ± 0.22^{c-d}
	20	64.29 ± 0.09^{a-c}	-1.28 ± 0.19^{e-f}	9.04 ± 0.45^{d-e}
	24	64.47 ± 0.21^{ab}	-1.39 ± 0.16^{e-f}	7.68 ± 0.92^{m}

Note. CT = cooking time (min). Data are expressed as mean ±SD; Different letters indicate statistically significant differences exist $p < 0.05$ for each column. Means that do not share a letter are significantly different. Tukey's test was applied with 95% simultaneous confidence intervals

Yellowness (b*) fell significantly in all three samples as cooking time increased ($p<0.05$). Parboiled MR297 dropped from 12.24 to 5.50, unparboiled MR297 from 5.96 to 1.17, and the commercial parboiled rice from 19.16 to 7.68. By 24 min, parboiled MR297 did not differ significantly from the commercial sample. The pattern follows what was seen for L* and a*, and the same explanation applies. Water uptake dilutes or masks the surface pigments, so the loss of yellowness reflects hydration rather than a separate colour-forming reaction. This runs opposite to several other starchy foods. In those products, extended heating causes darkening as free amino acids react with reducing sugars in Maillard-type browning (Sovia et al., 2026). The direction of colour change under heat may therefore depend on a food's amino acid and reducing sugar content rather than on starch gelatinisation alone.

Cooking Properties

Table 4 displays the variations in the grain size of various rice samples throughout the cooking process. The elongation ratio increased from 1.16 to 1.44 for parboiled rice MR297,

from 1.08 to 1.34 for unparboiled MR297, and from 1.17 to 1.46 for commercially parboiled rice MR297. Elongation reflects preferential water uptake and swelling along the length axis of the kernel. Starch granules face the least constraint from the surrounding cell-wall structure in that direction. Unparboiled rice showed slightly higher elongation in the early stages. This fits its faster initial moisture uptake relative to parboiled rice (see Section Moisture sorption), since the denser granule packing produced by parboiling slows water penetration at first. The elongation ratio change seen in this study is less than that of rice types cultivated in India (1.5-1.8) (Febina et al., 2023).

Table 4
The effects of cooking time on the cooking properties of MR297 parboiled rice

Rice	CT	Elongation Ratio	Moisture Sorption	l/b Ratio
Parboiled rice MR297	4	1.16 ± 0.06^{g}	0.43 ± 0.06^{i}	$3.71 \pm 0.24^{a-d}$
	8	1.24 ± 0.07^{e}	$0.65 \pm 0.03^{g-h}$	$3.58 \pm 0.32^{a-e}$
	12	$1.29 \pm 0.08^{d-e}$	$0.95 \pm 0.10^{e-f}$	$3.70 \pm 0.35^{a-d}$
	16	$1.36 \pm 0.08^{b-c}$	$1.12 \pm 0.03^{c-e}$	3.85 ± 0.29^{a}
	20	$1.36 \pm 0.07^{b-c}$	$1.28 \pm 0.06^{b-c}$	3.78 ± 0.30^{a}
	24	1.44 ± 0.10^{a}	1.63 ± 0.07^{a}	$3.76 \pm 0.32^{a-b}$
Unparboiled Rice	4	1.08 ± 0.06^{h}	$0.48 \pm 0.04^{h-i}$	3.41 ± 0.36^{e}
	8	1.16 ± 0.08^{g}	$0.82 \pm 0.01^{f-g}$	$3.30 \pm 0.27^{d-e}$
	12	$1.29 \pm 0.08^{d-e}$	1.32 ± 0.04^{b}	$3.45 \pm 0.27^{c-e}$
	16	$1.31 \pm 0.09^{b-d}$	1.55 ± 0.03^{a}	$3.48 \pm 0.31^{b-e}$
	20	$1.33 \pm 0.09^{b-d}$	1.57 ± 0.02^{a}	$3.49 \pm 0.29^{b-e}$
	24	$1.34 \pm 0.10^{b-d}$	1.57 ± 0.03^{a}	$3.44 \pm 0.36^{c-e}$
Commercially parboiled Malaysian rice	4	$1.17 \pm 0.04^{f-g}$	0.40 ± 0.03^{i}	$3.72 \pm 0.21^{a-c}$
	8	$1.23 \pm 0.04^{e-f}$	$0.50 \pm 0.13^{h-i}$	$3.59 \pm 0.22^{a-e}$
	12	$1.30 \pm 0.04^{c-e}$	$0.79 \pm 0.07^{f-g}$	$3.71 \pm 0.24^{a-c}$
	16	1.37 ± 0.04^{b}	$1.07 \pm 0.06^{d-e}$	3.84 ± 0.30^{a}
	20	1.37 ± 0.04^{b}	$1.26 \pm 0.08^{b-d}$	$3.75 \pm 0.26^{a-b}$
	24	1.46 ± 0.05^{a}	1.57 ± 0.10^{a}	$3.73 \pm 0.27^{a-b}$

Note. CT = cooking time (min). Data are expressed as mean ±SD; Different letters indicate statistically significant differences exist $p < 0.05$ for each column. Means that do not share a letter are significantly different. Tukey's test was applied with 95% simultaneous confidence intervals.

Moisture sorption increased from 0.430 to 1.633 for parboiled rice MR297, from 0.48 to 1.57 for unparboiled MR297, and from 0.40 to 1.58 for commercially parboiled rice MR297. Table 4 illustrates that the moisture sorption of the unparboiled sample shows a slower rate of increase after reaching 16 minutes of cooking. Conversely, the absorption rate of moisture in parboiled rice MR297 was slower than in unparboiled rice MR297. Parboiled rice absorbed more moisture than unparboiled rice by the end of cooking.

Continued heating disrupts the crystalline structure and hydrogen bonding of the starch granules, allowing water uptake to progress alongside gelatinisation (Gavahian et al., 2019). The slower uptake seen early on in parboiled rice fits its denser pre-gelatinised granule structure (Islam et al., 2004), which takes longer for water to penetrate. Once this structure starts breaking down under continued cooking, though, that same denser matrix may end up retaining more absorbed water at equilibrium, which would explain its higher sorption by 24 min.

Weight gain is a representation of the water that is absorbed by the starch of the grain that is capable of keeping water in the granules. This weight gain is estimated as hydration rates for various types of rice, including cultivars and ages. The increase in moisture sorption with increasing cooking time ($p < 0.05$) can be seen in Table 4. As noted above, unparboiled rice absorbed moisture more rapidly at the start of cooking, while parboiled MR297 and commercial parboiled rice absorbed moisture more gradually, yet parboiled rice ultimately absorbed more water than unparboiled rice by the final cooking time. The results indicated that the parboiling technique altered the starch properties in the grain, resulting in a delayed hydration and gelatinisation process compared to unparboiled rice.

Rice grains are measured in terms of their compared to their width, which is referred to as the L/B ratio. Among the three kinds of rice that were cooked, the L/B ratio ranged from 3.3 to 3.8. The l/b ratio does not exhibit a discernible trend as cooking time increases. This result indicates that length and width expand roughly proportionally during cooking rather than one dimension outpacing the other. However, the table shows that there were significant difference of l/b ratio between the three samples. The results indicated that the l/b ratio of unparboiled rice MR297 is smaller than the l/b ratio of both MR297 and the commercial parboiled rice ($p < 0.05$). The change in dimensions observed upon parboiling indicates a small shift in the l/b ratio of the cooked rice, rather than in cooking time itself.

Principal Component Analysis

PCA helped identify which variables best capture how cooked-rice quality shifts over cooking time (Figure 4). Springiness was excluded from the analysis. Its value stayed nearly constant across all treatments ($p > 0.05$; Table 1), so it added no meaningful variance to correlate against the other variables. The first two components together accounted for 87.4% of the total variance (PC1: 64.1%, PC2: 23.3%; Table 5). PC1 loaded most heavily on length, width, thickness, elongation, moisture sorption, and lightness (L*), with loadings between 0.32 and 0.33. Hardness and chewiness loaded negatively (-0.32 and -0.32; Table 6). PC1 therefore captures the overall cooking-time trajectory. As cooking progresses, the dimensional, moisture, and colour variables rise together while hardness and chewiness fall. PC2 was most strongly tied to l/b ratio (-0.53) and adhesiveness (-0.57), the two variables that most clearly distinguish unparboiled from parboiled samples (Table 6). PC3 explained only 5.2% of the variance and is not discussed further. Processing duration is

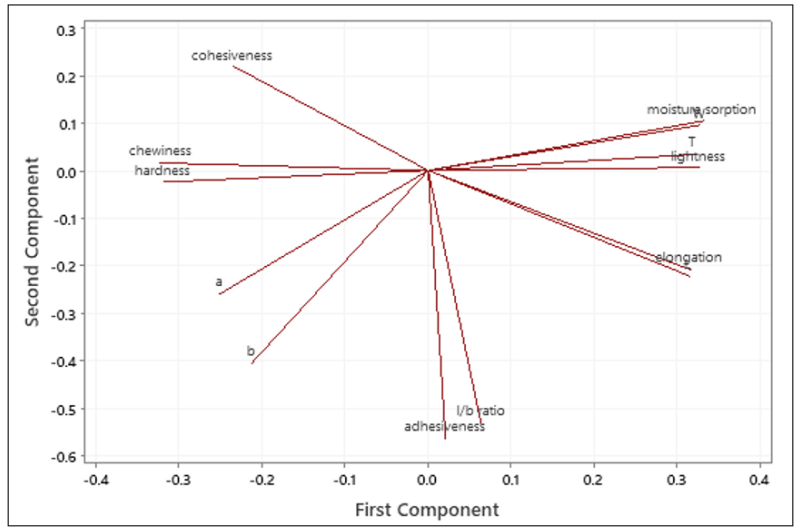


Figure 4. First and second component plots on the x and y axes for the (physical, textural, and cooking properties)

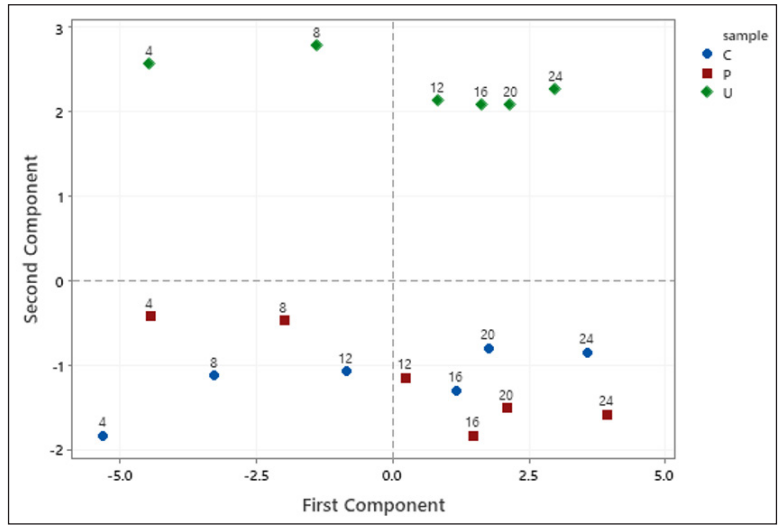


Figure 5. Score plot of cooked rice at different cooking times for the first and second principal components

similarly recognised as a critical determinant of physicochemical outcomes across other food matrices (Istrada et al., 2026).

The score plot (Figure 5) shows clear separation along both axes. Samples moved rightward along PC1 as cooking time increased, confirming it as the dominant cooking-time axis. Unparboiled rice samples clustered more tightly after 12 min. Both parboiled samples only began clustering from 16 min onward. Quality changes therefore appear to

Table 5
Eigen analysis of the Correlation Matrix

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**
Eigenvalue	8.339	3.030	0.674	0.428	0.289	0.123	0.059	0.031	0.013	0.009	0.004	0.000	0.0000
Proportion	0.641	0.233	0.052	0.033	0.022	0.009	0.005	0.002	0.001	0.001	0.000	0.000	0.000
Cumulative	0.641	0.875	0.926	0.959	0.982	0.991	0.996	0.998	0.999	1.000	1.000	1.000	1.000

Table 6
Eigenvectors

Variable	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**PC10**	**PC11**	**PC12**	**PC13**
Length	0.315	-0.222	-0.127	0.131	0.021	-0.036	0.214	0.215	0.250	-0.017	0.023	-0.069	0.810
Width	0.328	0.095	-0.040	0.360	-0.105	0.298	0.252	0.238	0.167	0.173	-0.013	0.632	-0.273
Thickness	0.320	0.033	-0.135	0.509	0.074	-0.057	-0.284	-0.640	0.048	-0.240	-0.245	-0.025	0.007
elongation	0.316	-0.209	-0.172	0.145	-0.025	-0.043	0.194	0.238	0.230	0.056	-0.021	-0.656	-0.471
l/b ratio	0.064	-0.533	-0.170	-0.272	0.224	-0.559	0.017	-0.139	0.195	0.125	-0.046	0.374	-0.176
moisture sorption	0.332	0.104	-0.017	0.189	0.033	-0.440	0.068	0.179	-0.742	0.024	0.246	0.017	0.004
Lightness (L*)	0.327	0.006	-0.229	-0.221	0.151	0.213	-0.780	0.296	0.021	0.104	0.124	0.034	0.003
a*	-0.251	-0.260	0.431	0.445	-0.352	-0.293	-0.392	0.315	0.144	-0.029	-0.027	0.028	-0.003
b*	-0.214	-0.407	-0.308	0.188	-0.289	0.271	-0.035	-0.281	-0.226	0.521	0.302	-0.060	0.063
hardness	-0.318	-0.024	-0.114	0.298	0.595	0.056	-0.010	0.236	-0.177	0.306	-0.504	-0.048	0.049
adhesiveness	0.021	-0.566	0.003	-0.074	-0.031	0.377	0.053	0.122	-0.365	-0.572	-0.204	0.087	-0.066
cohesiveness	-0.235	0.219	-0.702	-0.011	-0.440	-0.220	-0.027	0.193	-0.005	-0.195	-0.285	0.073	0.012
chewiness	-0.323	0.016	-0.258	0.295	0.391	-0.001	0.018	0.082	0.174	-0.389	0.627	0.051	-0.071

taper off earlier in unparboiled rice than in parboiled rice. Unparboiled MR297 sat in the upper part of the plot, while parboiled MR297 and commercial parboiled rice occupied the lower part. This confirms PC2 (l/b ratio, adhesiveness) as the axis separating parboiled from unparboiled samples. The convergence of parboiled samples along PC1 from 16 min onward matches the plateau in hardness and chewiness seen in Table 1 over the same window. Together, these support 16-20 min as the point past which further cooking produces only diminishing changes to most quality parameters, a useful benchmark for recommending a practical cooking time.

CONCLUSION

Cooking time significantly shaped the textural, physical, colour, and cooking properties of parboiled MR297 rice relative to its unparboiled and commercially parboiled counterparts. Extending cooking time consistently softened the grain and reduced its cohesiveness and chewiness. Grain dimensions, lightness, elongation, and moisture uptake all increased over the same range. Springiness and the l/b ratio remained stable regardless of cooking duration. Parboiled MR297 was consistently less sticky and less cohesive than unparboiled rice. It also reached a final size and brightness comparable to the commercial parboiled benchmark. Principal component analysis confirmed cooking duration as the dominant source of variation among samples. Parboiling status formed a secondary but consistent distinction based on l/b ratio and adhesiveness.

These findings provide the first cooking-time profile for parboiled MR297. Appropriately timed cooking brings its texture and appearance in line with an established commercial parboiled rice despite the lower milling yield of MR297. Textural softening plateaus while moisture uptake continues beyond this point. A cooking time of approximately 16 to 20 min is therefore recommended for parboiled MR297.

This study relied on instrumental and physicochemical measures only. Sensory and consumer acceptance were not assessed, so eating-quality conclusions are instrumentally inferred rather than taster-confirmed. Findings are based on a single paddy batch and one commercial benchmark, so they may not capture broader seasonal or supplier variability.

Future work should incorporate sensory evaluation to validate the recommended cooking time. Direct examination of compositional changes such as amylose and amylopectin ratio and starch crystallinity would confirm the structural mechanisms proposed here. Testing across multiple paddy batches and harvest seasons would also strengthen the generalisability of these findings.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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Impact of Soybeans-Shallots Intercropping on Physical Quality and Antioxidant Capacity of Shallots

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ABSTRACT

This research aims to investigate the impact of various soybean varieties, combined with the application of potassium nitrate (KNO_3), on the growth, yield, bulb characteristics, and antioxidant activity of shallots (*Allium cepa*) grown in an intercropping system. The study was conducted from May to August 2025 at Tohudan, Colomadu, Karanganyar, Central Java, Indonesia, at an altitude of 105 meters above sea level. The study used a factorial randomised complete block design consisting of three soybean varieties: Biosoy, Grobogan, and Dega, and three KNO_3 concentrations (0, 3, and 6 g L^{-1}). The observed parameters included soybean growth and yield attributes: plant height, number of pods, seed weight, and 100-seed weight. Observations of shallots included growth, bulb yield, colour characteristics, and antioxidant activity (DPPH radical-scavenging capacity, %RSA). The results showed significant effects of variety, KNO_3, and their interaction on the performance of both soybeans and shallots. Biosoy exhibited the highest growth and yield attributes among the soybean varieties tested and also improved shallot bulb productivity in the intercropping system. Intercropping with Biosoy, combined with a moderate KNO_3 application (3 g L^{-1}), enhanced bulb redness (a*) and chroma, indicating greater colour intensity and market quality. The highest antioxidant activity (94.94% RSA) was observed in the Biosoy without KNO_3 treatment, whereas higher KNO_3 levels tended to reduce antioxidant potential. These findings suggest that intercropping shallots with the Biosoy soybean variety and moderate KNO_3 fertilization optimizes both agronomic productivity and functional bulb quality, offering a sustainable approach to improving land-use efficiency in limited farmland areas.

Keywords: Antioxidant; intercropping; potassium; shallot; soybean

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INTRODUCTION

In 2022, the shallot area planted amounted to 184,984 hectares. The reduction in this amount was over 3 hectares in 2023. This is despite the fact that shallots are spices that are consumed nearly every day in Indonesian cuisine (Al Rosyid & Pratiwi, 2024). More than 85% of the soybean farmers in Indonesia still practice monoculture by having one crop in a piece of land. The drawbacks of this system are that it is susceptible to pests, diseases and weeds and relies heavily on the use of pesticides to safeguard crops. The monoculture of soybean planting in Indonesia is 79%, and 21% of soybean farming is intercropping (Didorenko et al., 2021) (Central Bureau of Statistics, 2024). The dangers of land scarcity, increasing food pressure and climate change are becoming all the more complicated in agricultural problems. On the island of Java, land shortage is evident, with agricultural land being transformed to non- agricultural land at an annual rate of 12% (Durán-Sandoval et al., 2023). To effectively ensure that these issues are mitigated, intercropping has been suggested as a solution to the challenges of land use and agricultural output. Intercropping advantages include: (a) the greatest possible use of space, light, water and nutrients, (b) the reduction of pest and disease attacks, (c) a decrease of soil erosion, since the various root systems absorb water, nutrients and light, (d) an enhanced use of water, since different absorption patterns are employed, (e) increased farmer income, as different plants lessen the likelihood of crop failures, (f) increased biodiversity of agricultural land, (g) greater climate-change resilience, since different absorption patterns are used, and (h) better soil health, since more organic matter is accumulated and soil structure is improved (Toker et al., 2024).

The corn-soybean system is one of the most popular ways of applying intercropping that provides a sustainable system to smallholder farmers having limited land resources (Xu et al., 2020). Being a legume, soybean develops a symbiotic relationship with Rhizobium bacteria that help the fixation of atmospheric nitrogen, thus increasing the availability of nitrogen to the soybean crop and intercrop partners (Abd-Alla et al., 2023). Soybean is an important food product because it is a major source of vegetable protein that contains all the amino acids and is a major raw material in the tofu and tempeh industries. Consequently, it is important to develop sustainable soybean farming to propel the food self-sufficiency program in Indonesia.

The required nitrogen for the shallots (*Allium cepa var. aggregatum L.*) growth may be provided by the soybean root nodules. The spacing usually applied in intercropping systems is the soybean spacing of 40 x 30 cm between the rows of the soybeans, with the shallots in between the rows of the soybean to allow maximum land utilisation. Shallots are an extremely popular product in Indonesia, and can be used both as a spice in the kitchen and as a component of traditional medicine (Yofananda et al., 2021). Shallots contain bioactive compounds like quercetin, flavonoids, saponins, tannins, glycosides, polyphenols and alkaloids. Shallots even contribute to their antioxidant, antibacterial, antiviral, antidiabetic,

and anti-inflammatory properties (Anggarani & Amalia, 2022). Sustainable production of shallots is, however, limited by environmental conditions, particularly water availability, as optimum production is achieved during dry seasons with adequate irrigation.

Shallots can also be used to provide bioactive compounds; besides, they can be used to facilitate intercropping systems as they have the capacity to reduce pests that attack soybean plants. The intercropping of soybean with corn, oil palm and cassava has been investigated before (Xu et al., 2020; Wagino et al., 2024); but only a few studies have investigated soybean-shallot intercropping. Therefore, the research proposal will test the influence of intercultural sowing of soybean and shallot to the treatment of nitrate fertiliser on the physical and antioxidant qualities of shallots.

MATERIALS AND METHODS

Materials and Experimental Site

This experiment was conducted at the Tohudan experimental site, Colomadu, Karanganyar Regency, Central Java, Indonesia (105 m above sea level) between May and August 2025. The mean temperature was 29-32 °C, and the total area of the experiment was around 300-400 m². This study used three types of soybean, which included Biosoy, Grobogan and Dega. Shallot bulbs were planted between the rows of the soybean. Basal fertilisers were applied using chicken manure and NPK fertiliser (15:15:15), and potassium nitrate (KNO_3) was used as a treatment fertiliser.

Experimental Design

The study was set up using a randomised complete block design (RCBD). With a factorial treatment structure and three replications. The type of soybean (Biosoy = B, Grobogan = G, and Dega = D) was the first factor, and the dosage of KNO_3: K0 (0 g per plant), K1 (7.5 g per plant) and K2 (15 g per plant) was the second one. There were nine treatments that were applied: Biosoy + no Potassium; Biosoy + Potassium 7.5 g per plant; Biosoy + Potassium 15 g per plant. Grobogan - does not contain Potassium; Grobogan Potassium 7.5 g/plant; Grobogan + Potassium 15 g/plant. Dega × - potassium; Dega + Potassium 7.5 g/plant; Potassium 15 g/plant (Table 1).

Table 1
Treatment Codes

Treatment	Without Potassium Nitrate	Potassium Nitrate7.5 g per Plant	Potassium Nitrate 15 g per Plant
Biosoy variety	BK0	BK1	BK2
Grobogan variety	GK0	GK1	GK2
Dega variety	DK0	DK1	DK2

Experimental Procedure

Land preparation consisted of ploughing and weeding, and later 20 t ha^{-1} of chicken manure and 50 t ha^{-1} of NPK (15:15:15) fertiliser. The spacing of the soybean was 40 x 20 cm with two seeds per hole, with a depth of 3cm. Each plot had 28 soybean plants and was 120 x 120 cm. The transplantation of shallot bulbs between soybean rows was done after 25 days of soybean planting at a spacing of 40 cm with 18 shallot plants per plot. The treatments were 30 and 40 days of planting, where KNO_3 was used in two equal doses. Crop management activities like irrigation, weeding, pest and disease control were performed on a need basis. The soybeans were harvested 83-85 days after planting.

Growth and Yield Parameters

The measurements of the soybeans were the number of pods per plant, weight of each plant, weight of each plot, number of seeds per plant, weight of each plant and weight of 100 seeds. The observations were made of shallots in terms of height of the plants, the number of bulbs per cluster, weight of fresh bulbs per cluster, weight of dry bulbs per cluster and the diameter of the bulb.

Colour Analysis

The colour of the shallot bulbs was measured with the help of a chromameter on the basis of the CIELAB system. Measurements were made of the brightness (L*), the red-green axis (a*) and the yellow-blue axis (b*). Chroma (C*), hue angle (h°), and total colour variation (ΔE) were calculated. This was measured three times, and the values are reported as the standard deviation and the mean.

Antioxidant Activity (DPPH Assay)

The 2, 2-diphenyl-1-picryl-hydrazine (DPPH) test was used to measure antioxidant capacity. The DPPH solution was made 0.1 mM in methanol, and the necessary concentration of shallot extract was added to the solution. This solution was then left in the dark at room temperature, and after 30 minutes, the absorbance of the solution was measured at 517 nm on the UV-Vis spectrophotometer. The scavenging activity percentage (RSA) was determined, and the IC_{50} was determined by regression analysis in the case of a series of extract concentrations.

Statistical Analysis

Analysis of variance (ANOVA) was used to analyse all variables use SPSS software. The comparison of the treatment means was done using the Duncan Multiple Range Test (DMRT) at a significance level of 5%.

RESULTS AND DISCUSSION

Soybean Performance

The performance of soybean varieties in terms of yield was highly affected by the use of KNO_3 (Table 2). Biosoy recorded a higher number of pods, weight of the pod and seed per plant than Grobogan and Dega. This difference could be attributed to the genetic difference in uptake potential and assimilate partitioning. The use of potassium nitrate enhanced pod filling, weight of 100 seeds and productivity. This is consistent with Torabian et al., (2021) who noted that potassium plays a vital role in the photosynthetic process, enzyme activation, and assimilate transport. The stimulating activity of KNO_3 may also be attributed to the enhancement of nitrogen assimilation, since nitrate is a biogenic compound and a signal which triggers protein synthesis (Soujanya et al., 2024). Similar findings were reported by Menalled et al., (2021), who found that as the uptake capacity increased, so did seed yield. These results indicate that diversity is important, and equal K fertilisation is significant for maximising soybean yield. The best outcomes for the Biosoy soybean variety were achieved with the application of 7.5 g of K fertiliser.

The use of Biosoy as an intercrop and the application of KNO_3 resulted in a significant enhancement in the growth and yield parameters (plant height, number of bulbs, fresh and dry bulb weight) of shallot (Table 2). Allium plants need potassium as it controls carbohydrate metabolism, sulfur assimilation, and water balance (Farghly et al., 2021). The increase in the size and weight of the bulb during fertilisation with KNO_3 is similar to other reports such as Etana et al. (2019), which propose that the application of K leads to an increase in dry matter in garlic and onions. The microenvironment of shallots is favourable when Biosoy is grown with them, compared with Grobogan and Dega, leading to denser

Table 2
Growth and yield of soybeans in different varieties and KNO_3 treatments

Treatments	Plant Height (cm)	Pods per Plant	Seed Weight per Plant (g)	100-seed Weight (g)
Biosoy + KNO3 0 g	32.11^{b}	18.2^{b}	12.8^{b}	15.6^{a}
Biosoy + KNO3 7.5 g	44.29^{a}	22.5^{a}	16.9^{a}	15.8^{a}
Biosoy + KNO3 15 g	48.04^{a}	21.8^{a}	17.4^{a}	15.9^{a}
Grobogan + KNO3 0 g	23.75^{c}	14.3^{c}	9.6^{c}	14.3^{b}
Grobogan + KNO3 7.5 g	25.24^{c}	15.1^{c}	10.2^{c}	14.5^{b}
Grobogan + KNO3 15 g	37.53^{b}	17.4^{b}	13.7^{b}	14.8^{b}
Dega + KNO3 0 g	19.22^{c}	12.1^{c}	8.3^{c}	13.5^{c}
Dega + KNO3 7.5 g	25.14^{c}	13.8^{c}	9.1^{c}	13.9^{c}
Dega + KNO3 15 g	42.05^{ab}	16.2^{b}	12.4^{b}	14.1^{c}

Note. Data represent mean values (n = 3). Distinct superscript letters in the same column denote statistically significant differences (DMRT, $p \leq 0.05$)

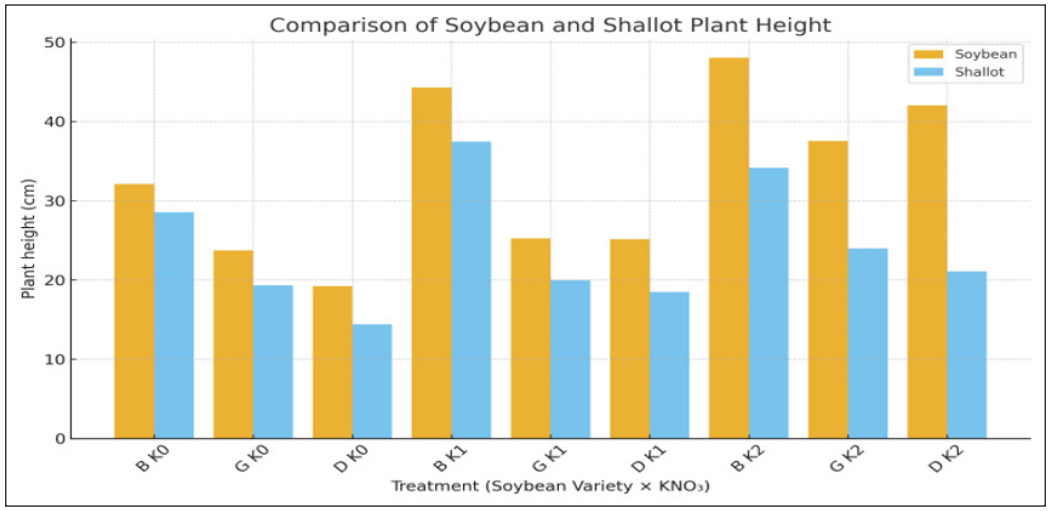


Figure 1. Height of soybean and shallot plants in different varieties and KNO_3 treatments (*B K0: Biosoy + KNO_3 0 g per plant; G K0: Grobogan + KNO_3 0 g per plant; D K0: Dega + KNO_3 0 g per plant; B K1: Biosoy + KNO_3 7.5 g per plant; G K1: Grobogan + KNO_3 7.5 g per plant; D K1: Dega + KNO_3 7.5 g per plant; B K2: Biosoy + KNO_3 15 g per plant; G K2: Grobogan + KNO_3 15 g per plant; D K2: Dega + KNO_3 15 g per plant)

canopies. Intercropping relies on the interspecific complementarity in light capturing and the efficacy of nutrient use (Yang et al., 2023). Moderate application of KNO_3 (K1); middle concentrations were the best, and the balance of nutrients to grow bulbs and reach their quality should be taken into account (Toker et al., 2024).

The height of the soybean plants was always greater than that of shallots, and thus the shade was different among the varieties (Figure 1). The competitive advantage of taller soybean plants reduced the height of shallots in Grobogan and Dega, and Biosoy allowed relatively good growth of shallots. Competitive advantage of a crop is the ability of a commodity or agricultural product to compete and out-compete other analogous products in the market. This is done by cost efficiency, distinctive characteristics (taste or nutrition) and even resistance to pests. Intercropping systems are known to have vertical asymmetry. Interception of light has been particularly essential in plants that need light (Allium plants) (Alam et al., 2019). Low levels of light suppress photosynthetic activities and the growth of carbohydrates, thereby preventing the growth of the bulbs. The same has been observed in the context of cereal legume intercropping where canopy structure plays a considerable role in compatibility and productivity across the system (Brooker et al., 2015; Soujanya et al., 2024). Such findings imply the significance of planting soybean varieties that do not grow a vigorous canopy when planted with shallots (Table 3).

In addition to the productivity of individual crops, the other parameter of intercropping that is crucial is the land equivalent ratio (LER), which is the efficiency of land use relative to the sole crop. The yield patterns used in this study, though, did not provide the actual LER,

Table 3
Growth and yield of shallots in the intercropping treatment of soybeans and KNO_3

Treatments	Plant Height (cm)	Bulbs per Hill	Fresh Bulb Weight (g)	Dry Bulb Weight (g)	Bulb Diameter (mm)	Moisture Content (%)
Biosoy + KNO3 0 g	28.57^{b}	4.8^{b}	19.6^{b}	12.3^{b}	24.8^{a}	82.1^{a}
Biosoy + KNO3 7.5 g	37.47^{a}	6.2^{a}	25.7^{a}	15.6^{a}	25.4^{a}	82.4^{a}
Biosoy + KNO3 15 g	34.14^{a}	6.0^{a}	26.2^{a}	16.1^{a}	25.6^{a}	82.3^{a}
Grobogan + KNO3 0 g	19.35^{c}	3.5^{c}	14.2^{c}	8.9^{c}	23.1^{b}	81.2^{ab}
Grobogan + KNO3 7.5 g	19.98^{c}	3.7^{c}	15.1^{c}	9.2^{c}	23.4^{b}	81.0^{ab}
Grobogan + KNO3 15 g	23.99^{bc}	4.3^{bc}	17.8^{b}	10.4^{bc}	23.9^{b}	81.5^{ab}
Dega + KNO3 0 g	14.40^{d}	2.8^{c}	12.4^{c}	7.2^{c}	22.6^{b}	80.4^{b}
Dega + KNO3 7.5 g	18.48^{cd}	3.4^{c}	13.6^{c}	8.1^{c}	22.8^{b}	80.7^{b}
Dega + KNO3 15 g	21.10^{c}	3.9^{c}	15.9^{bc}	9.8^{bc}	23.2^{b}	81.1^{b}

Note. Data represent mean values (n = 3). Distinct superscript letters in the same column denote statistically significant differences (DMRT, $p \leq 0.05$)

but there is a possibility of higher efficiency in land use, especially when using the Biosoy x K1 combination. It has been emphasised in the prior literature that LER values more than 1 mean that intercropping systems are better than monocropping systems (Bhattacharya, 2019; Maclaren et al., 2023). Therefore, implementing LER analysis in future studies will strengthen the ecological and economic justification for soybean-shallot intercropping.

The observed poor growth of shallot in Grobogan and Dega might be attributed to the canopy covering of the shallot canopy that limits the amount of photosynthetically active radiation (PAR) to the canopy. Photosynthesis rate, carbon assimilation and the increase of the bulb are limited directly by the decrease of PAR. Biosoy with less competitive canopy architecture allows more light to penetrate, hence allowing shallot to grow. Similar mechanisms have been reported in other systems, and canopy structure is a key factor in establishing intercrop compatibility and productivity in cereal-legume systems (Major et al., 2022).

Colour Attributes of Shallot Bulbs

Colour attributes (L*, a*, b*, chroma, and hue angle) were very much affected by variety and addition of KNO_3 application (Table 4). Intercropping biosoy with moderate KNO_3 (K1) resulted in higher redness (a*) and chroma, which is a measure of pigment strength. Potassium enhances anthocyanins and flavonoids due to an increase in the level of carbohydrates and stabilisation of pigment production (Vincent-Caboud et al., 2019; Yan et al., 2025). The alterations in the hue angles indicate the alterations in the pigment composition, particularly anthocyanins and quercetin derivatives, which constitute the

Table 4
Colour attributes of shallot bulbs under soybean and KNO_3 intercropping treatments

Treatment	L* (Mean ± SD)	A* (Mean ± SD)	B* (Mean ± SD)
Biosoy + KNO3 0 g	49.26 ± 4.06	28.62 ± 4.74	−13.97 ± 1.47
Biosoy + KNO3 7.5 g	42.59 ± 7.73	34.25 ± 3.25	−15.05 ± 0.46
Biosoy + KNO3 15 g	47.79 ± 4.35	29.16 ± 6.23	−12.86 ± 1.48
Grobogan + KNO3 0 g	53.46 ± 4.05	25.06 ± 3.53	−12.66 ± 1.63
Grobogan + KNO3 7.5 g	53.47 ± 0.89	19.20 ± 1.09	−10.41 ± 0.71
Grobogan + KNO3 15 g	34.88 ± 0.46	37.14 ± 1.33	−15.30 ± 0.42
Dega + KNO3 0 g	46.60 ± 1.07	26.98 ± 2.64	−12.77 ± 0.77
Dega + KNO3 7.5 g	38.67 ± 3.89	33.78 ± 0.65	−13.98 ± 0.45
Dega + KNO3 15 g	46.47 ± 3.67	32.89 ± 5.98	−15.00 ± 0.16

**Note*. Data represent mean values (n = 3). Distinct superscript letters in the same column denote statistically significant differences (DMRT, $p \leq 0.05$)



Figure 2. Harvested shallot samples from an intercropping system with soybean

major part of shallot bulb colour (Zhang et al., 2018). The accumulation of pigments can also be significantly affected by environmental and nutritional parameters; one of them is potassium, which may enhance the stability of anthocyanins and inhibit oxidative breakdown (Hasanuzzaman et al., 2018). Since the colour of the bulb directly depends on consumer preference and marketability, K supply in intercropping systems is important in enhancing quality (Figure 2).

Other Allium species have been reported to have similar patterns. Fertilisation with potassium and nitrogen increases the size and yield of bulbs in shallots and garlic and has a tendency to decrease the quantity of flavonoids and antioxidant potential (Chernukha et al., 2022). The results of this experiment confirm that shallots also act similarly, which implies the need to take fertilisation strategies into account to have the most commercial traits and health-promoting properties.

Antioxidant Activity

The effects of the soybean variety, the dosage of potassium nitrate and the interactions between them on the antioxidant activity of shallot bulbs (percentage reduction in soybean activity, a.k.a. percent reduce activity, or percent RSA) were significant. Biosoy showed by far the best antioxidant activity in zero KNO_3 (Biosoy × K0), meaning that there was a good natural genetic ability to produce phenolic and flavonoid compounds. Recent reports testify that genotypic variation is a powerful regulator of the antioxidant potential in Allium crops due to variation of quercetin, flavonols, sulfur-based compounds, and secondary metabolites (Yang et al., 2023).

It was also observed that there was an evident decreasing tendency as KNO_3 content of all varieties increased. This pattern is consistent with new findings that increased nitrogen levels tend to lower phenolic levels by the carbon-nitrogen trade-off process, in which plants use more carbon in growth-related processes instead of secondary metabolism (Supriyadi et al., 2020). High levels of nitrates increase vegetative biomass and water content, and in this case, the dilution effect decreases the phytochemicals to unit dry mass ratio (Olsovska et al., 2024).

The results of the current study agree with the observations of other Allium species, in which over-fertilisation reduces the level of flavonoids and antioxidant ability (Hasanuzzaman et al., 2018). On the other hand, moderate or even lower nutrient intakes are likely to increase stress-responsive biosynthesis in phenolic pathways, increasing the antioxidant activity (Ninkuu et al., 2025). In this way, the elevated antioxidant activity of Biosoy (B) in the absence of potassium and B K1 (KNO_3 7.5 g) would probably indicate the presence of weak stresses on the nutrients that cause the synthesis of protective secondary metabolites.

The varietal differences were also involved. Biosoy could sustain a good amount of antioxidants in shallots in contrast to Grobogan and Dega. This aligns with the fact that various intercropping partners develop diverse below-ground interactions (root exudates, N fixation, rhizosphere signalling), which are capable of controlling biosynthesis of companion crop secondary metabolites (Brooker et al., 2015). Both intercropping of the legumes with high N fixation and high N fixation increase the amount of nitrogen available in the soil but also produce competition or facilitation effects that change the metabolic allocation in shallots.

Regarding the functional food, the present results underline that the antioxidant content of shallots is extremely sensitive to fertilisation methods. The antioxidant activity was found to be relatively high in moderate KNO_3 (7.5 g), though the highest values were always at zero KNO_3 (Figure 3). This pattern is similar to the latest reviews that suggest a possibility to increase the phytochemical density of vegetables by reducing excessive fertilisation (Olsovska et al., 2024). Therefore, KNO_3 inputs have to be maximised to achieve the yield as well as to maintain the nutritional and functional characteristics of shallot bulbs.

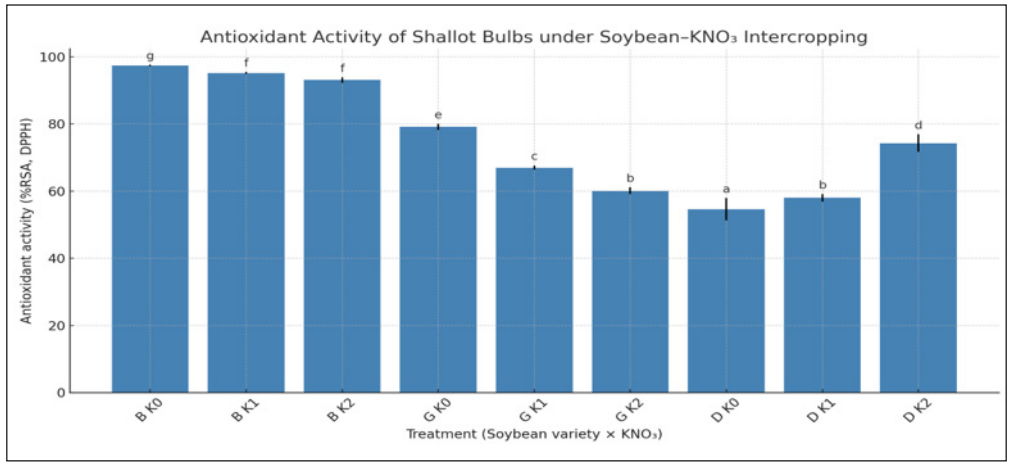


Figure 3. Antioxidant activity (%RSA, DPPH assay) of shallot bulbs grown under soybean-KNO_3 intercropping treatments. Values are presented as mean ± SD (n = 4). Different letters above the bars signify statistically significant differences based on Duncan's Multiple Range Test at $p \leq 0.05$. Antioxidant activity in shallots is driven by flavonoids, phenolics, and sulfur-containing compounds and is strongly influenced by genotype and nutrient availability

Overall, these results confirm. Letters above the bars indicate the statistically significant differences on the basis of Duncan Multiple Range Test, p 0.05. The moderate fertilisation by using Biosoy soybean and moderate KNO_3 is a viable method to regulate the yield-to-quality of functional and moderate functional quality, and minimal fertilisation adds to the antioxidant potential further. This agroecological feedback correlates with modern ideas of sustainable intensification, in which the mutual optimisation of nutrient efficiency and functional food properties is attained (Yofananda et al., 2021).

CONCLUSION

This study shows that the yield and quality of soybean in intercropping with shallot are very much reliant on the soybean variety and the quantity of KNO_3 to be applied. Biosoy could exhibit consistent good performance in soybean and grow a canopy structure that increased light penetration and consequently the growth of shallots and yield of the bulbs. The interactions between variety and fertilisers had a strong impact on bulb colour, and the moderate KNO_3 had a positive effect on pigment intensity in combination with Biosoy. Bioactivity was also not the same across the variety × fertiliser interactions, with more KNO_3 in Biosoy and Grobogan and less in Dega, showing that varieties react differently to biochemical reactions. In general, soybean-shallot intercropping increased the efficiency of the land, crop yield, and the nutritional value of shallots, and Biosoy + moderate KNO_3 (K1) was the best agronomic combination. The findings highlight the necessity of integrating appropriate variety selection and equalising nutrient management in the

attainment of sustainable intensification, improved crop quality and improved resiliency in smallholder systems.

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CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest.

HIGHLIGHTS

- Intercropping soybeans with shallots significantly increases the yield and quality of the tubers.
- Biosoy variety combined with moderate KNO_3 is producing the best agronomic performance and quality.
- The antioxidant activity of shallot bulbs decreased with increasing KNO_3 supply, indicating a trade-off between yield and phytochemical content.

AUTHOR CONTRIBUTIONS

Conceptualisation	:	Sumarmi
Methodology	:	Sumarmi, Avisema Sigit Saputro, Fadilah Husnun
Formal analysis	:	Sumarmi, Avisema Sigit Saputro
Investigation	:	Sumarmi; Edy Listanto
Writing - original draft preparation	:	Sumarmi, Avisema Sigit Saputro, Fadilah Husnun
Writing - review and editing	:	Sumarmi, Fadilah Husnun; Edy Listanto
Supervision	:	Sumarmi, Edy Listanto

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Spatial-Based Analysis of Rice Field Drought Levels in West Sumatra Province

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ABSTRACT

Drought is a natural disaster that significantly impacts the agricultural sector, water availability, and public welfare. This study aims to identify drought in rice fields in West Sumatra using the Standardised Precipitation Index (SPI) and Normalised Difference Drought Index (NDDI) methods. The SPI is used to determine drought based on rainfall deviations from normal conditions over a given period. The NDDI is used to measure drought conditions based on soil moisture and vegetation conditions. The novelty of this study lies in the spatial-temporal integration of SPI and NDDI for West Sumatra, an approach rarely applied in Indonesia, as well as in linking rainfall variability with paddy vegetation responses for early detection of agricultural drought. The SPI analysis identified 370 drought events, affecting a total of 2,377.40 ha, or 1.01% of the total rice fields, in July 2023, while the NDDI analysis revealed a much wider drought coverage, reaching 198,772.11 ha (84.76%) categorised as severe to extremely dry. The difference in results between the two indices indicates that statistically adequate rainfall does not necessarily reflect sufficient groundwater availability to support vegetation growth. These findings emphasise the importance of integrating meteorological indicators with land and crop conditions for more accurate agricultural drought monitoring. These findings support adaptive planting patterns and efficient irrigation management, enhancing agricultural resilience to drought and contributing to SDG 2 (Zero Hunger) and SDG 6 (Clean Water and Sanitation).

Keywords: Climate variability, drought, GIS, NDDI, rice field, SPI

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INTRODUCTION

According to the Intergovernmental Panel on Climate Change (IPCC) report (2023), there is an increase in the intensity, frequency, and duration of various extreme weather events, including droughts, which are caused by climate change and impact the agricultural sector (IPCC, 2023). Global climate change is causing changes in rainfall patterns in many countries, including Indonesia (Irsyad & Oue, 2020). Shifts in rainfall patterns also cause shifts in the rainy and dry seasons in a region (Avia, 2019). These seasonal shifts impact the availability of air, which indicates the onset of drought (Lesik et al., 2020).

Drought occurs almost every year in Indonesia and increases sharply during El Niño conditions, leading to decreased agricultural production (Novianti, 2019). West Sumatra Province also faces the challenge of drought, despite being known for its high rainfall. However, the uneven distribution of rainfall in the region and the impact of climate change make certain areas in this province vulnerable to drought (Hermawan & Komalaningsih, 2008). In 2022, 87 hectares of rice fields in West Sumatra Province experienced drought, according to data from the Regional Technical Implementation Unit of the Food Crop and Horticulture Protection Centre of West Sumatra Province. The dry season occurred in eight regencies and cities: Pesisir Selatan Regency, Agam Regency, Solok Regency, Sijunjung Regency, 50 Cities, Tanah Datar Regency, West Pasaman Regency, and Solok City (Agusri, 2022). Based on this description, a study of the extent and distribution of drought is needed to mitigate the drought disaster in West Sumatra Province.

The SPI is a very useful tool for monitoring meteorological drought that uses rainfall data in its analysis (Dutta et al., 2015). The simple calculation, minimal data usage (only rainfall) and flexibility of the SPI make this method recommended by the World Meteorological Organisation (WMO) as the preferred indicator for monitoring meteorological drought (Hayes et al., 2011; Purnamasari et al., 2017). The SPI method is widely used because it is relatively easy, can provide good comparative values, and is able to identify levels of wetness and dryness simultaneously (Mckee et al., 1993; Sunusi & Auliana, 2025). This method measures rainfall deficits at different time scales. The average time selected to determine drought using this method is a monthly period scale, which is 1, 3, 6, 12, 24, or 48 months. Previous research has shown that the SPI method can well identify drought conditions in an area, such as research by Shah in 2015. The results of this study show that the drought obtained by the SPI method is almost the same as the actual drought situation (Shah et al., 2015). Furthermore, research by Yusman in 2025 showed that the SPI method can identify the level and distribution of drought that occurred in Agam Regency (Yusman et al., 2025).

The SPI requires long-term rainfall data with high temporal resolution to achieve superb results. Areas with limited or low-quality data will face difficulties in calculating the SPI accurately (Wicaksanti et al., 2019). The weakness of the SPI method is that the input data is

only rainfall. When a lot of data is missing, it will give wrong results, because the program will provide output for every input given (WMO & GWP, 2016). However, SPI alone cannot adequately represent the spatial response of drought on rice vegetation because it is based solely on precipitation anomalies. This limitation creates a research gap in drought assessment, particularly for agricultural areas where drought impacts are expressed not only through rainfall deficits but also through changes in vegetation condition and surface water availability. Therefore, integrating SPI with a remote sensing-based drought index such as NDDI is necessary to provide complementary information on meteorological drought and its spatial manifestation in rice fields. Therefore, to obtain more accurate drought information, a drought analysis was conducted using the Normalised Difference Drought Index (NDDI) method. The NDDI method is a drought index that, in its analysis, is based on vegetation density and water availability simultaneously, thus providing more comprehensive results for drought-prone areas (Rahman et al., 2017). The NDDI method was developed by Yingxin Gu in 2007 and combines two parameters: the wetness index (NDWI) and the vegetation index (NDVI) (Gu et al., 2007). The NDDI drought index is more effective in describing the level of drought in a region due to its greater accuracy, particularly in reflecting vegetation response to drought stress (Luqman et al., 2022).

Drought analysis using the SPI and NDDI is crucial for rice paddies, given that rice plants are highly sensitive to changes in water availability. The SPI can detect meteorological drought, which affects rainfall as the primary source of irrigation, while the NDDI captures the impact of drought on rice vegetation spatially. Unlike prior studies limited to district-level or single-index drought analysis, this study integrates SPI and NDDI indices at the provincial scale to capture meteorological and vegetation-based drought interactions. The combination of these two indices can provide a more comprehensive picture of drought dynamics in rice paddies, supporting more precise data-driven mitigation and agricultural planning efforts. Identifying rice fields affected by drought is crucial for providing early warning, reducing farmer losses, and ensuring national food security. This information enables the government and farmers to take preventive and mitigating measures such as adjusting cropping patterns, providing drought-resistant varieties, and allocating emergency aid to mitigate the impacts of more severe droughts. This study is also aligned with the Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger) and SDG 6 (Clean Water and Sanitation), by supporting climate-resilient agriculture, food security, and more effective irrigation and water-resource management.

The drought analysis methods frequently used in Indonesia are the SPI and SPEI, which display two sets of data: a map of areas likely to experience drought and a drought index graph for each province in Indonesia, displaying the upper and lower limits of the model (Adiningsih et al., 2016; Sutanto, 2017) states that several models have been developed and applied in Indonesia using low- and medium-resolution remote sensing data, either

singly or in combination. The accuracy of models developed to date using GMS, MTSAT, TRMM, QMorph, MODIS, AVHRR, Landsat-7, SPOT-4, and ALOS data is generally adequate at the national to district levels, with an average coefficient of variation ranging from 60% to 80%. Many drought studies have been conducted using a single index. In this study, the SPI and NDDI indices were combined. This study aims to identify drought in rice fields in West Sumatra Province using the Standardised Precipitation Index (SPI) and Normalised Difference Drought Index (NDDI) methods.

MATERIALS AND METHODS

Research Area

West Sumatra Province, located on the island of Sumatra, Indonesia (0°54'-3°30'S, 98°36'-101°53'E), covers 4,212,043.87 hectares across 19 regencies/cities, as shown in Figure 1. It borders North Sumatra Province to the north, Riau and Jambi Provinces to the east, Bengkulu Province to the south, and the Indian Ocean to the west. Based on 2022 data from the Geospatial Information Agency (BIG), processed in ArcGIS, West Sumatra Province covers 234,504.93 hectares of rice paddy land. The SPI requires long-term rainfall data with high temporal resolution

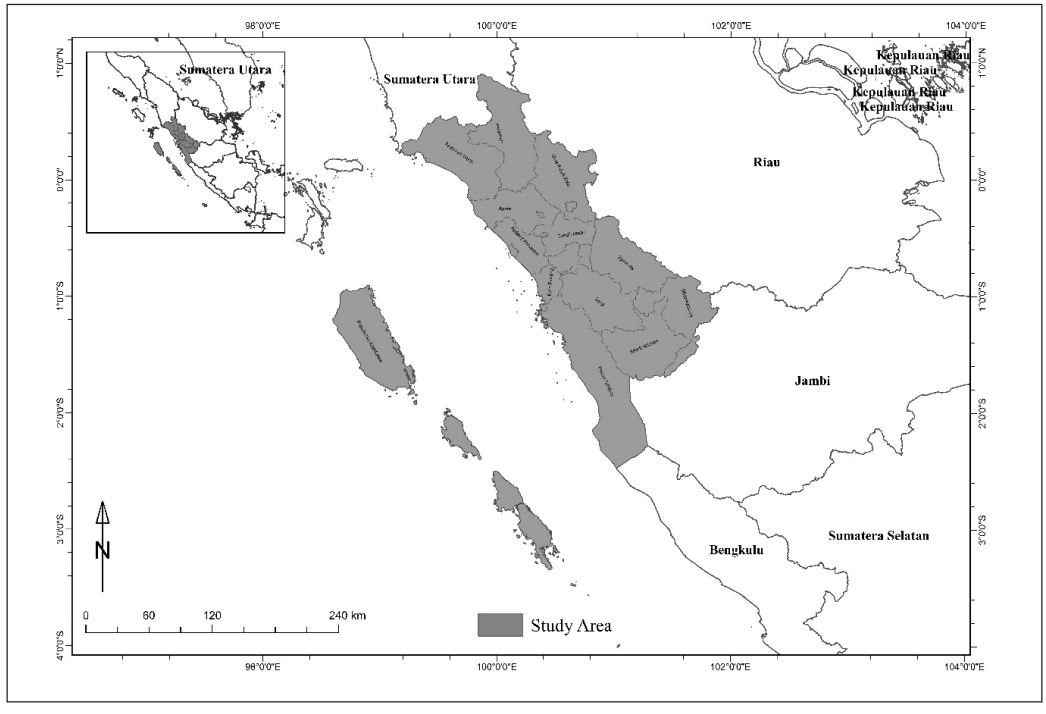


Figure 1. Map of research locations in West Sumatra province

Data Source

This study uses secondary data, namely rainfall data for the last 30 years (1994-2023). This data was obtained from the West Sumatra Provincial Water Resources and Construction Agency and the West Sumatra Meteorology, Climatology, and Geophysics Agency (https://dataonline.bmkg.go.id/). Landsat 8 satellite imagery was obtained from the USGS website (http://earthexplorer.usgs.gov/). Administrative boundary data and rice field land use data for West Sumatra Province were obtained from the Geospatial Information Agency (BIG) website (http://tanahair.indonesia.go.id).

Rainfall Data Completeness Analysis

In this study, two methods were used to complete missing rainfall data: the Reciprocal Method and rainfall data from NASA POWER (Prediction of Worldwide Energy Resources). The reciprocal method was used to complete missing data at each rainfall station with up to two months of missing data. Rainfall data from the observation stations were used as the primary data source. When rainfall observations were unavailable for more than two consecutive months, the missing observations were supplemented using rainfall data from NASA POWER to maintain the continuity of the rainfall time series for SPI calculation. The NASA POWER rainfall data used were obtained from the website https://power.larc.nasa.gov/data-access-viewer/. The variables used in the reciprocal method were rainfall data and the distance between the nearest station and the station where the missing rainfall data was sought, using the Equation 1.

$$\frac{\frac{Pa}{Dxa^2}+\frac{Pb}{Dxb^2}+\ldots+\frac{Pn}{Dxn^2}}{\frac{1}{Dxa^2}+\frac{1}{Dxb^2}+\ldots+\frac{1}{Dxn^2}} \quad [1]$$

Rainfall Data Consistency Analysis

Rainfall data consistency analysis aims to verify the accuracy of field data. Rainfall data consistency is tested using the Double Mass Curve method, which compares the monthly cumulative rainfall from station A (on the y-axis) with the cumulative rainfall from a reference station (on the x-axis). The reference station used is the average of several stations closest to the station whose consistency is being tested (Maulina et al., 2022). These cumulative values are then plotted on a Cartesian x-y coordinate system, and the resulting curve is examined to observe changes in slope (trend). These changes in slope can indicate discrepancies in the data that need to be analysed to ensure the consistency of rainfall data. The consistency value ranges from 0 to 1; the closer the value is to 1, the more consistent the data quality is.

Drought Analysis Using the Standardised Precipitation Index (SPI) Method

The SPI index value can be calculated based on the gamma distribution, which is defined as the following frequency or probability function in Equations 2 to 5:

$$G(x)=\int_0^x g(x)=\frac{1}{\beta^{\alpha}T(\alpha)}\int_0^x x^{a-1}\,e^{-x/\beta}dx \quad [2]$$

where is:

$$\alpha=\frac{1}{4A}\left(1+\sqrt{1+\frac{4A}{3}}\right) \quad [3]$$

$$A=ln(\bar{x})-\frac{\sum ln(x)}{n} \quad [4]$$

$$\beta=\frac{\bar{x}}{a} \quad [5]$$

Where T (a) is the gamma function, while n is the number of observed rainfall data, $\bar{x}$ is the average monthly rainfall (mm/month), and x is the monthly rainfall data (mm/month). The following is a classification of drought levels based on the SPI index (Table 1).

Image Selection for Drought Analysis Using the Normalised Difference Drought Index (NDDI) Method

The imagery used in this study is Landsat 8, which has two sensors: the Operational Land Imager (OLI) and the Thermal Infrared Sensor (TIRS). Launched in 2013, the Landsat 8 satellite has continued and enriched the uninterrupted multispectral record of the Earth's surface since the inception of the Landsat program in 1972 (Ihlen, 2019). The imagery used in the

Table 1
Classification of SPI drought index values

SPI Index Value	Drought Classification
≤ -2	Extremely dry
-1.99 s/d -1.50	Severely dry
-1.49 s/d -1.0	Moderately dry
-0.99 s/d 0.99	Near normal
1.00 s/d 1.49	Moderately wet
1.50 s/d 1.99	Severely Wet
2	Extremely Wet

Source: Sunusi & Auliana, 2025

NDDI drought analysis was selected based on the months with the lowest rainfall and the highest incidence of SPI drought events. This was done to observe drought conditions in West Sumatra Province during the period of lowest rainfall, a critical time for assessing drought severity. By selecting imagery from drier months, the analysis can focus more on the impact of water shortages on vegetation and soil surface conditions.

In addition to rainfall, cloud cover is also an important factor in image selection. Clouds can impair image quality by reducing contrast and obscuring ground surface details, thus affecting the accuracy of data interpretation (Zhu & Woodcock, 2012). To minimise this impact, images with minimal cloud cover are prioritised for use in analysis. Landsat 8 is equipped with Quality Assessment (QA) bands that enable cloud identification and masking so that only cloud-free areas are used in the analysis (Foga et al., 2017).

Image Pre-Processing

Prior to calculating NDVI, NDWI, and NDDI, the selected Landsat 8 OLI/TIRS imagery underwent several pre-processing steps to ensure the quality and consistency of the satellite data. First, the Landsat 8 Level 2 Surface Reflectance product was selected to minimise atmospheric effects and provide surface reflectance data suitable for spectral index calculations. Second, cloud and cloud-shadow masking was performed using the Quality Assessment (QA_PIXEL) band to remove pixels affected by clouds, cloud shadows, and other unwanted atmospheric conditions. Third, the relevant spectral bands were extracted, namely Band 4 (Red), Band 5 (NIR), and Band 6 (SWIR-1), which were required for the calculation of NDVI and NDWI (Table 2). Fourth, the imagery was clipped to the boundary of West Sumatra Province to limit the analysis to the research area and reduce unnecessary data processing. Finally, the pre-processed bands were used to calculate NDVI and NDWI, which were subsequently combined to derive the NDDI values. All image pre-processing and spectral index calculations were performed using ArcGIS software.

Drought Analysis Using the Normalised Difference Drought Index (NDDI) Method

The analysis of drought severity levels is carried out by utilising remote sensing technology because changes in the reflectance of crops reflect physiological and morphological

Table 2
Landsat 8 OLI and TIRS spectral bands resolution

Name	Spectral Bands	Wavelength (µm)	Spatial Resolution (m)
Band 4	Red	0.636-0.673	30
Band 5	Nir	0.851-0.879	30
Band 6	Swir-1	1.566-1.651	30

Source: Ihlen, 2019

responses to water stress experienced by the plants (Patil et al., 2024). The Normalised Difference Vegetation Index (NDVI) is the result of a normalised reflectance comparison between the near-infrared (NIR) band and the red band, which is widely used to monitor vegetation conditions on the Earth's surface. NDVI serves as a simple yet effective quantitative indicator to describe the density and health condition of vegetation through remote sensing methods, as in Equations 6 and 7.

$$NDVI = \frac{\rho NIR - \rho RED}{\rho NIR + \rho RED} \quad [6]$$

In the case of Landsat 8, NDVI is calculated using Equation 7.

$$NDVI = \frac{Band\ 5 - Band\ 4}{Band\ 5 + Band\ 4} \quad [7]$$

NDVI values are influenced by spatial factors such as climate, soil characteristics, and topography; thus, ecosystems with higher productivity exhibit distinct radiometric responses compared to less productive ones. Temporal variations in NDVI-based drought indices were derived from satellite imagery and analysed using GIS tools to evaluate drought severity across the study area.

The Normalised Difference Water Index (NDWI) is an important spectral index used to monitor water content and surface moisture conditions. It is particularly effective in distinguishing water bodies from vegetation and soil by exploiting the reflectance differences between the green and near-infrared (NIR) spectral bands. In this study, NDWI was calculated using satellite imagery to assess spatiotemporal variations in surface water availability and moisture conditions within the study area, as in Equation 8.

$$NDWI = \frac{\rho NIR - \rho SWIR}{\rho NIR + \rho SWIR} \quad [8]$$

NDWI for Landsat 8 was calculated using Equation 9.

$$NDVI = \frac{Band\ 5 - Band\ 6}{Band\ 5 + Band\ 6} \quad [9]$$

The NDDI is an index used to identify drought levels. The NDDI drought index value is obtained by combining two parameters: the Normalised Difference Water Index (NDWI)

and the Normalised Difference Vegetation Index (NDVI). The NDDI index value can be calculated using Equation 10.

$$NDDI = \frac{NDVI\text{-}NDWI}{NDVI+NDWI} \quad [10]$$

NDDI is a more sensitive indicator for drought compared to the two indicators above, namely NDVI and NDWI. Elevated NDDI values signify dry conditions, coinciding with reduced NDVI and NDWI values. In contrast, lower NDDI values represent areas with adequate water availability, where both NDVI and NDWI increase, indicating well-hydrated and vigorous vegetation (Patil et al., 2024). Classification of NDDI Drought Index Values is presented in Table 3 and the data processing flowchart in Figure 2.

Table 3
Classification of NDDI Drought Index Values

Class	NDDI	Category
1	-1 to 0	No drought
2	0 to 0.1	Starting to dry
3	0.1 to 0.2	Moderate
4	0.2 to 0.3	Severe
5	0.3 to 0.4	Extreme
6	0.4 to 1	Very extreme

Source: Artikanur et al., 2022

Analysis of Potentially Drought-Prone Rice Fields Using the SPI and NDDI Methods

After obtaining the Standardised Precipitation Index (SPI) and Normalised Difference Drought Index (NDDI) drought index values, the data were used to create a drought

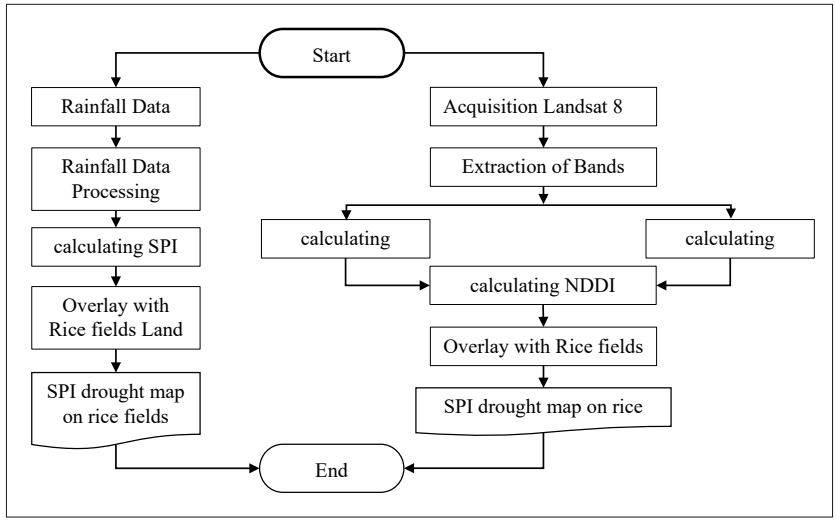


Figure 2. Data processing flowchart

distribution map, showing affected areas with varying degrees of drought. The SPI drought distribution map was created spatially using ArcGIS software.

The drought-analysis month was selected based on the minimum monthly rainfall observed during the 1994-2023 period. Monthly rainfall records were first analysed to identify the month with the lowest long-term rainfall condition. The selected month was subsequently used to represent the period with the greatest potential for meteorological drought and to generate the spatial distribution of SPI in West Sumatra Province.

The inverse distance weighted (IDW) interpolation method was used to interpret the SPI drought index values to obtain a map of the drought distribution in West Sumatra Province. The IDW interpolation method was applied to estimate SPI values at unsampled locations based on the weighted contribution of surrounding rainfall stations. The weighting decreases with increasing distance from the prediction location, such that closer stations have a greater influence on the estimated value. The interpolation was performed in ArcGIS using the specified power parameter and neighbourhood setting. The resulting continuous SPI surface was subsequently classified according to the adopted SPI drought categories.

The interpolated SPI drought map was subsequently overlaid with the rice-field spatial dataset using a GIS overlay operation. The rice-field dataset was first projected to the same coordinate reference system as the SPI raster. The SPI raster was then clipped and intersected with the rice-field polygons to identify the spatial extent and proportion of rice fields within each drought severity class.

The NDDI drought map was created using two important indicators: vegetation condition (NDVI) and water availability (NDWI). Through processing satellite imagery data using ArcGIS, a map was produced showing drought-affected areas in West Sumatra. This map is then combined with rice field data so that an overview of the distribution of drought in rice fields in each region is obtained.

Next, a comparative analysis of the SPI and NDDI results is carried out to see more comprehensive drought results. The inverse distance weighted (IDW) interpolation method was used to interpolate the SPI drought index values to obtain a map of the drought distribution in West Sumatra Province. The Inverse Distance Weighted (IDW) interpolation method was selected because the SPI values were obtained from discrete rainfall stations, and the spatial interpolation assumes that rainfall conditions at locations closer to an observation station are more similar than those at more distant locations. IDW assigns greater weights to nearby stations and progressively lower weights to more distant stations. This method was considered appropriate for the present study because the objective was to generate a spatial distribution map of SPI values based on the available rainfall station network. Compared with geostatistical methods such as Kriging, IDW does not require the estimation and fitting of a variogram or prior modelling of spatial autocorrelation. Therefore, considering the objective of the study and the characteristics of the available rainfall station data, IDW was selected as a practical method for spatial interpolation of

SPI values. Then the map is overlaid with data on rice fields. So, a map of the distribution of SPI drought in rice fields is obtained.

Validation of Drought Maps

The reliability of the drought maps derived from the SPI and NDDI methods was assessed using an independent reference dataset representing actual drought conditions. The validation dataset was not used in the calculation of the SPI or NDDI indices to maintain the independence of the assessment. Spatial matching was conducted by assigning the reference observations to the corresponding rice-field polygons or validation units, while temporal matching was performed by selecting drought maps corresponding to the same observation period as the reference data. Only observations with consistent spatial and temporal coverage were included in the validation analysis.

The drought classification derived from the SPI and NDDI maps was compared with the corresponding reference drought conditions. These metrics were used to quantify the agreement between the mapped drought classes and the independent reference data.

The validation results were subsequently used to evaluate the reliability of the SPI and NDDI approaches in identifying drought-prone rice fields in West Sumatra Province. Differences between the mapped drought conditions and the reference observations were also examined to identify areas where the indices may overestimate or underestimate actual drought conditions.

RESULTS

Rainfall

The rainfall data used in this study come from 47 rainfall stations distributed throughout the regencies/cities in West Sumatra Province, with an observation period of 30 years (1994-2023). An analysis of the monthly average rainfall shows a consistent seasonal pattern, with rainfall peaks occurring in November at 384 mm and the lowest values in July at 192 mm. This condition indicates that West Sumatra has a monsoon climate characterised by a rainy season at the end of the year and a dry season in the middle of the year, as shown in Table 4.

Rainfall Data Consistency

It is a crucial step in ensuring the accuracy and reliability of data analysis before use in drought analysis based on the Standardised Precipitation Index (SPI). Data consistency is crucial because inconsistencies can arise from recording errors, equipment failure, differences in measurement methods, or changes in environmental conditions, which can potentially introduce bias and lead to incorrect interpretations. The results of the consistency test are shown in Table 5.

Table 4
Monthly precipitation from 1994-2023

No	Station Name	Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	Batang Kapas	265	212	243	229	175	220	200	226	244	310	355	311
2	Batu Busuk	256	190	307	313	252	268	244	283	291	339	445	371
3	Bendung Batang Hari	275	254	275	337	268	186	157	206	218	253	377	329
4	Bendung Koto Tuo	260	233	261	269	251	282	236	292	321	357	456	371
5	Canduang	215	171	257	291	181	137	137	158	168	221	279	262
6	Danau Diatas	254	182	223	284	240	187	124	171	166	220	300	310
7	Durian Simpai Silago	237	223	276	302	197	181	164	177	205	273	371	309
8	Ganggo Mudiak	282	230	347	402	284	271	210	329	326	377	447	411
9	BMKG Padang Panjang	322	298	350	408	248	212	199	262	298	359	445	459
10	Gumarang	276	238	304	313	239	196	191	252	290	332	422	369
11	Gunung Nago	311	221	326	301	297	308	272	333	388	431	520	396
12	Jalan Balantai	220	188	245	231	175	182	154	179	182	232	285	274
13	Kampung IV	231	201	290	304	229	330	221	304	286	351	391	313
14	Kandang IV	394	346	489	573	394	317	324	367	406	472	568	542
15	Kasang	324	285	334	329	265	306	255	332	375	387	451	395
16	BMKG Sumatera Barat	389	304	371	442	297	275	249	354	402	385	521	455
17	Komplek PU Sedasi	298	329	319	346	244	170	173	199	203	252	351	331
18	Koto Baru Piruko	225	195	249	285	204	151	155	170	200	232	365	300
19	Ladang Padi	310	253	359	375	349	328	311	305	337	435	527	423
20	Limau Manih	245	162	264	257	220	248	195	265	272	300	395	351
21	Lintau Buo	212	183	201	208	149	121	90	138	135	204	234	240
22	Lubuak Napar	357	289	438	472	296	294	239	349	348	388	516	484
23	Lunang	209	213	231	208	216	170	151	202	202	250	301	253
24	Manggopoh	224	167	219	240	191	163	165	254	264	279	343	309
25	BMKG Teluk Bayur	324	251	316	305	286	340	319	336	395	392	527	472
26	BMKG Minang Kabau	305	300	361	316	253	305	282	327	359	403	481	420
27	Muaro Labuh	125	116	130	146	113	86	89	104	111	121	161	142
28	Muaro Tantang	258	195	273	318	234	210	191	253	236	295	349	328
29	Nanggalo Tarusan	279	209	289	272	222	250	222	277	277	350	416	364
30	Padang Aro	259	246	304	276	208	206	165	183	200	217	309	265
31	Padang Sidodang	198	184	252	272	193	170	144	159	190	234	316	261
32	Paraman Talang	357	299	410	451	331	245	233	341	356	367	456	422
33	Rao	247	183	235	240	189	145	143	184	206	262	323	296
34	Saniang Baka	254	220	248	256	192	143	135	194	209	215	289	288
35	Santok	265	212	256	223	181	202	231	280	287	366	426	323
36	Sijunjung	230	202	216	279	190	146	136	165	169	196	292	250
37	Silapiang	368	287	376	401	263	200	226	325	324	363	449	390

Table 4 (*continued*)

No	Station Name	Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
38	Sontang	177	166	170	230	141	132	110	154	192	209	303	293
39	Suko Mananti	247	192	306	350	278	261	242	315	302	358	432	353
40	Suliki	244	210	245	304	161	153	136	174	243	286	298	314
41	Sumani	181	140	174	185	131	106	95	156	142	193	237	250
42	Sungai Ipuh	234	204	233	231	159	115	105	129	189	201	298	202
43	Surantiah	265	198	213	186	155	168	189	214	223	266	334	308
44	Tanjung Pati	219	171	210	261	145	120	125	149	186	201	302	254
45	Tapan Bakir	357	317	354	298	302	292	233	285	330	425	461	386
46	Tarusan	315	213	278	257	204	219	194	256	280	321	371	363
47	Ujung Gading	336	320	395	395	241	245	278	425	419	488	564	478
	Average	269	226	286	302	226	212	192	245	263	306	384	340

Table 5
Rainfall data consistency test values

No	Station Name	R^2 Value	No	Station Name	R^2 Value
1	Batang Kapas	0.9977	25	BMKG Teluk Bayur	0.9976
2	Batu Busuk	0.9973	26	BMKG Minang Kabau	0.9927
3	Bendung Batang Hari	0.9986	27	Muaro Labuh	0.9816
4	Bendung Koto Tuo	0.9948	28	Muaro Tantang	0.9979
5	Canduang	0.9958	29	Nanggalo Tarusan	0.9996
6	Danau Diatas	0.9956	30	Padang Aro	0.9921
7	Durian Simpai Silago	0.9954	31	Padang Sidodang	0.9952
8	Ganggo Mudiak	0.9964	32	Paraman Talang	0.9989
9	BMKG Padang Panjang	0.9846	33	Rao	0.9796
10	Gumarang	0.9959	34	Saniang Baka	0.9673
11	Gunung Nago	0.9951	35	Santok	0.9844
12	Jalan Balantai	0.9959	36	Sijunjung	0.9747
13	Kampung IV	0.9985	37	Silapiang	0.9935
14	Kandang IV	0.9999	38	Sontang	0.9982
15	Kasang	0.9981	39	Suko Mananti	0.9961
16	BMKG Sumatera Barat	0.9998	40	Suliki	0.9761
17	Komplek PU Sedasi	0.9930	41	Sumani	0.9993
18	Koto Baru Piruko	0.9947	42	Sungai Ipuh	0.9914
19	Ladang Padi	0.9977	43	Surantiah	0.9835
20	Limau Manih	0.9937	44	Tanjung Pati	0.9910
21	Lintau Buo	0.9798	45	Tapan Bakir	0.9955
22	Lubuak Napar	0.9996	46	Tarusan	0.9981
23	Lunang	0.9460	47	Ujung Gading	0.9950
24	Manggopoh	0.9617			

Standardised Precipitation Index (SPI) Drought Index

The SPI drought analysis is conducted monthly, based on 30 years of rainfall data (1994-2023). SPI calculations are performed monthly to identify rainfall variability and more accurately detect drought periods. An SPI value <-1 indicates drought conditions, with severity varying from moderately dry to extremely dry depending on the magnitude. The following shows the number of SPI drought events from 1994 to 2023 (Table 6).

Spatial Drought Distribution Based on the SPI Method in Rice Fields

The drought distribution map was created to identify affected areas during the observation period. This map displays the spatial distribution and intensity of drought, allowing identification of areas with the highest severity (Yusman et al., 2025). This information is obtained from the SPI drought index calculation, which is then visualised in spatial maps to provide a more comprehensive picture of drought distribution. By integrating paddy field land use data in West Sumatra, covering an area of 234,504.93 ha, with the SPI drought distribution map for July 2023 using an overlay technique, information was obtained regarding the spatial variation in drought impacts (Figure 3).

The map in Figure 3 illustrates the distribution of drought in West Sumatra Province based on the SPI Index values for July in 2014, 2017, 2020, and 2023. July was chosen as the focus of the analysis due to the high frequency of drought events during the 1994-2023 period, which coincides with the dry season or the transition phase leading to the peak of the dry season in most parts of Indonesia, including West Sumatra. During this period, rainfall generally decreases significantly compared to previous months, increasing the potential for drought. The results of this analysis allow for the identification of impacted areas in paddy fields in each district/city, which is presented in detail in Table 7.

Table 6
Number of drought events based on SPI method analysis 1994-2023

Month	Drought Level			Amount
	Extremely Dry	Severely Dry	Moderately Dry	
January	22	32	93	147
February	59	70	152	281
March	18	26	86	130
April	15	17	66	98
May	31	56	164	251
June	51	80	181	312
July	80	90	200	370
August	70	69	125	264
September	73	73	97	243
October	91	60	79	230
November	6	12	48	66
December	22	23	54	99

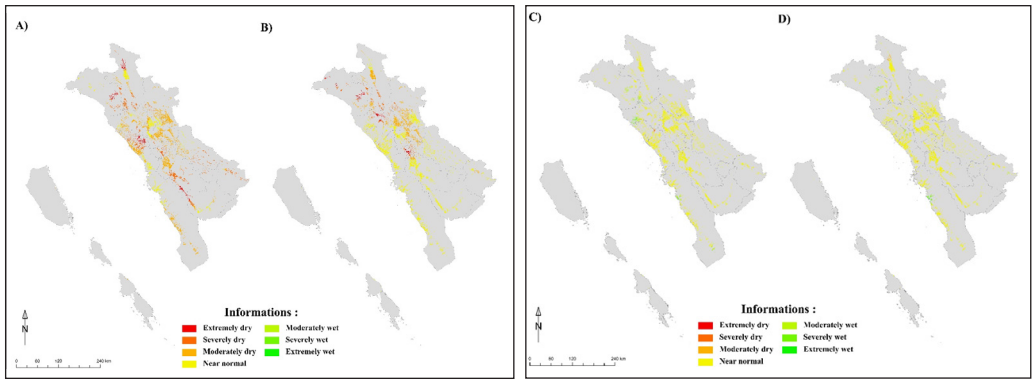


Figure 3. Spatial drought distribution of SPI in West Sumatra (A) 2014; (B) 2017; (C) 2020 and (D) 2023

Table 7
SPI Drought Area in Rice Fields in July 2023

No	Regency/City	Drought Level					
		Severely Dry (ha)	Moderately Dry (ha)	Near Normal (ha)	Moderately Wet (ha)	Severely Wet (ha)	Extremely Wet (ha)
1	Agam	0	0	27,885.49	65.18	0	0
2	Dharmasraya	0	0	4,405.08	518.46	0	0
3	Kepulauan Mentawai	0	0	294.57	0	0	0
4	Bukittinggi City	0	0	537.82	0	0	0
5	Padang City	6.45	340.56	6,596.90	10.57	0	0
6	Padang Panjang City	0	0	668.00	0	0	0
7	Pariaman City	0	0	2,088.51	0	0	0
8	Payakumbuh City	0	0	3,215.59	0	0	0
9	Sawahlunto City	0	0	1,528.70	0	0	0
10	Solok City	0	0	1,053.27	0	0	0
11	Lima Puluh Kota	0	0	25,462.00	0	0	0
12	Padang Pariaman	0	0	23,850.44	1,097.55	0	0
13	Pasaman	0	2,030.39	19,348.34	0	0	0
14	Pasaman Barat	0	0	7,537.60	1,118.11	688.15	0
15	Pesisir Selatan	0	0	21,591.83	1,167.22	1,812.27	287.87
16	Sijunjung	0	0	12,442.86	0	0	0
17	Solok	0	0	31,834.66	0	0	0
18	Solok Selatan	0	0	10,026.75	5.55	0	0
19	Tanah Datar	0	0	24,988.19	0	0	0
	Total	6.45	2,370.95	225,356.60	3,982.64	2,500.42	287.87

Spatial Drought Distribution Based on the NDDI Method in Rice Fields

The NDDI-based drought distribution map was created to identify the areas most affected by drought during the observation period. The NDDI considers the relationship between the vegetation index (NDVI) and the wetness index (NDWI), thus providing a more accurate spatial depiction of drought levels (Luqman et al., 2022). The distribution map is visualised as a colour gradient representing drought categories, ranging from normal to very extreme. Thus, the NDDI map shows not only the spatial distribution but also the intensity of drought in each region. Therefore, it can be used as a reference in formulating drought mitigation strategies in West Sumatra Province. The drought distribution map of West Sumatra Province, analysed using the NDDI method, is presented in Figure 4.

Based on Figure 4, the spatial distribution of drought in West Sumatra Province, analysed using the Normalised Difference Drought Index (NDDI), shows significant variations in drought severity. Areas dominated by green represent normal conditions with adequate moisture availability, while those coloured yellow to orange depict severe to extreme droughts that have the potential to hamper agricultural productivity. Meanwhile, areas coloured red indicate very extreme conditions with potentially serious impacts.

The analysis results show that the drought, based on the NDDI index, in July 2023 had a significant impact on rice fields in various regencies/cities in West Sumatra Province. Integrating the NDDI distribution map with the rice field map yields more comprehensive information, showing variations in drought severity across regions. This spatial-temporal information is crucial because it provides an overview of the drought dynamics experienced by rice fields during the observation period, as shown in the following Table 8.

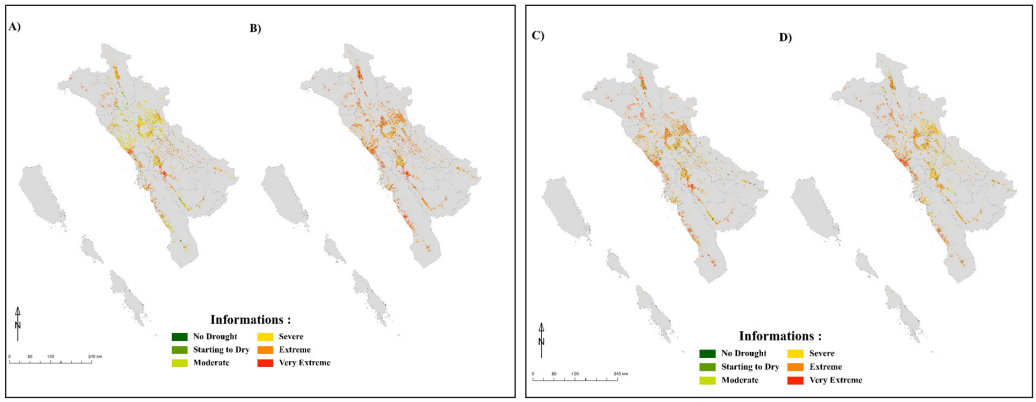


Figure 4. Spatial drought distribution of NDDI in West Sumatra (A) 2014; (B) 2017; (C) 2020; and (D) 2023

Table 8
NDDI drought extent in rice fields in July 2023

No	Regency/City	Area based on Drought Level (ha)					
		Very Extreme	Extreme	Severe	Moderate	Starting to Dry	No Dry
1	Agam	4,381.77	12,903.88	7,801.89	1,579.56	619.80	663.77
2	Dharmasraya	548.65	1,823.54	1,219.57	403.31	251.45	677.03
3	Kepulauan Mentawai	5.39	55.86	155.18	21.88	7.52	48.74
4	Bukittinggi City	193.79	188.92	91.36	27.95	15.73	20.08
5	Padang City	2,282.17	2,075.88	1,481.41	487.85	278.07	349.09
6	Padang Panjang City	117.37	326.52	191.04	25.16	5.58	2.33
7	Pariaman City	731.39	642.28	418.19	108.35	63.61	124.70
8	Payakumbuh City	258.45	1,224.04	1,248.48	274.66	117.98	91.98
9	Sawahlunto City	35.26	381.69	792.66	175.37	67.17	76.57
10	Solok City	156.58	243.61	393.29	119.90	63.56	76.34
11	Lima Puluh Kota	1,378.85	8,223.94	12,345.66	2,165.47	738.74	609.34
12	Padang Pariaman	8,799.41	8,603.63	5,127.69	1,118.64	573.39	725.23
13	Pasaman	5,282.67	6,974.47	5,309.21	1,991.66	926.02	894.69
14	Pasaman Barat	3,377.29	3,850.77	1,419.32	358.27	158.58	179.64
15	Pesisir Selatan	7,678.25	8,446.08	5,999.23	1,430.21	681.40	623.99
16	Sijunjung	668.04	3,022.05	5,038.83	1,954.07	962.61	797.26
17	Solok	3,660.62	9,905.65	12,207.14	3,124.39	1,177.19	1,759.67
18	Solok Selatan	1,501.39	4,402.76	2,918.43	616.40	319.60	273.70
19	Tanah Datar	2,351.10	8,275.75	9,633.75	2,280.30	1,000.75	1,446.54
	Total	43,408.45	81,571.33	73,792.33	18,263.40	8,028.74	9,440.68

Comparison of Drought Using the SPI and NDDI Methods

Based on the drought analysis using the Standardised Precipitation Index (SPI) in July 2023, rice fields in West Sumatra Province were generally classified as being in the normal category. However, a total of 2,377.40 ha of rice fields experienced drought, consisting of 6.45 ha classified as severely dry and 2,370.95 ha as moderately dry. However, this contrasts with the results of the NDDI drought analysis, which indicated drought conditions categorised as "moderate to very extreme." This discrepancy between the SPI and NDDI analysis results relates to land and water resource management in agricultural areas.

Uneven rainfall distribution requires the support of technical irrigation channels capable of distributing water regularly and measurably. Limited irrigation networks and infrastructure result in insufficient water supply, especially during periods of prolonged rainfall deficit. Without adequate irrigation infrastructure, rainwater that should be optimally utilised for agricultural needs is lost as surface runoff without being fully utilised by plants.

The discrepancy between SPI and NDDI should not necessarily be interpreted as an inconsistency between the two methods, but rather as an indication that they represent different dimensions of drought. SPI primarily reflects meteorological drought based on rainfall anomalies, whereas NDDI captures the response of vegetation and surface moisture conditions. Consequently, rice fields may remain in a normal meteorological condition according to SPI while experiencing water stress detected by NDDI because of differences in irrigation access, soil moisture conditions, topography, land management, and crop growth stage. This finding highlights the importance of integrating meteorological and remote-sensing indicators to obtain a more comprehensive understanding of agricultural drought conditions.

Recommendations for Drought Disaster Mitigation in Rice Fields

The integration of SPI and NDDI provides useful information for supporting drought management in rice-growing areas. SPI can be used to identify meteorological drought and provide an early indication of rainfall deficits, while NDDI can provide spatial information on the response of vegetation and surface moisture conditions at the agricultural field scale. The combined information can therefore support the prioritisation of drought interventions by identifying areas where rainfall deficits coincide with vegetation and surface moisture stress. Areas consistently identified as drought-prone can be prioritised for irrigation scheduling, water allocation, rehabilitation of irrigation infrastructure, and water conservation measures. In addition, NDDI-based spatial information can support local authorities in identifying rice fields that require closer monitoring during dry periods and can complement conventional rainfall-based drought monitoring.

In areas classified as mild to moderate drought, mitigation measures can be implemented through adjusting cropping patterns based on climate-based planting calendars, utilising organic mulch to reduce evaporation rates, and constructing water conservation microstructures such as rorak, infiltration ditches, and small dams. This approach is not only able to maintain optimal soil moisture but also extends water availability during the dry season, thus supporting the continuity of the plant growth phase (Wu et al., 2024).

Meanwhile, in areas classified as experiencing severe to extremely dry (extreme) drought, more intensive interventions are needed, such as the construction of reservoirs, water reservoirs, or retention dams to capture and store rainwater runoff during the rainy season. This infrastructure serves as a water reserve that can be gradually utilised during prolonged dry periods and is expected to increase the resilience of rice production in rain-fed areas (Dangnga et al., 2019). Furthermore, for drought-affected rice fields that still have irrigation systems but are damaged, rehabilitation and revitalisation of irrigation networks are necessary to ensure efficient and equitable water distribution. Integrating water conservation technology, infrastructure improvements, and the implementation of

adaptive cultivation practices is key to building a drought-resilient agricultural system, particularly in the face of the challenges of climate change and extreme weather variability.

DISCUSSION

Based on Table 4, the climate classification of (Anam et al., 2023; Oldeman & Frère, 1982), months with rainfall ≥ 200 mm are categorised as wet, 100-200 mm as humid, and < 100 mm as dry. The analysis shows that the average monthly rainfall in West Sumatra during the 1994-2023 period generally falls within the wet category, except for July, which is on the threshold of transitioning to the humid category. Quite high spatial variability was identified in July, with several stations recording wet conditions, while three other stations—Lintau Buo (90 mm/month), Muaro Labuah (89 mm/month), and Sumani (95 mm/month)—showed dry conditions. These findings indicate that, although July is generally wet to humid, certain areas experience significant rainfall deficits. This rainfall distribution pattern shows significant spatial and temporal variability. The analysis reveals a gradual decline in accumulated rainfall since May, reaching its lowest point in July, indicating the onset of the dry season, marked by a consistent decrease in rainfall intensity. This situation directly impacts water availability for irrigation, potentially reducing agricultural productivity, particularly for commodities sensitive to water deficits.

The results of the consistency test (Table 5) show that rainfall data from all stations have a very good level of reliability, indicated by the coefficient of determination (R^2) value, which is generally close to 1. According to (Maulina et al., 2022), the closer the R^2 value is to 1, the higher the data consistency, while a value close to 0 indicates data inconsistency. Based on the analysis results from 47 rain gauge stations, the average R^2 value from all stations was 0.9912. This value reflects high consistency, so it can be concluded that there were no significant changes in the quality of rainfall data at each station during the observation period.

Table 6 above presents the number of drought events based on the SPI Index, grouped into three levels of severity: Extremely dry, Severely dry, and Moderately dry, during the January-December period of 1994-2023. The data demonstrate variations in drought intensity and frequency throughout the year, clearly illustrating the dry season pattern in the study area. The analysis shows that the highest number of drought events occurred in July, with a total of 370 events, consisting of 80 Extremely dry events, 90 Severely dry events, and 200 moderately dry events. Besides July, the months with a relatively high frequency of drought were June (312 events), August (264 events), and September (243 events). This pattern indicates that the mid-year period is a critical phase with a higher risk of drought compared to other months. This phenomenon aligns with the characteristics of Indonesia's tropical climate, where the peak dry season generally occurs in the middle of the year and is characterised by a significant decrease in rainfall, including in West Sumatra.

Based on the SPI analysis, drought conditions in West Sumatra show interannual variation. In July 2014 and 2017, several regions, particularly Pasaman and West Pasaman Regencies, experienced high-intensity drought, as indicated by the predominance of red on the SPI map (Figure 3). This condition indicates a significant rainfall deficit compared to normal conditions. Conversely, other regions during the same period remained within the normal category with relatively stable rainfall. Meanwhile, hydrological conditions in July 2020 and 2023 tended to be better, with most areas of West Sumatra Province falling within the normal category based on the SPI index. This finding indicates interannual climate dynamics influencing rainfall patterns and drought vulnerability in the region.

The results of the temporal NDDI drought analysis method for July 2014, 2017, 2020, and 2023 indicate spatially fluctuating drought dynamics (Figure 4). 2017 was recorded as the period with the most severe drought, indicated by a significant increase in areas categorised as extreme to very extreme in various districts/cities. These conditions were likely influenced by regional climate anomalies and may partly reflect differences in irrigation access and local water management. Conversely, conditions in 2023 were relatively better, marked by a decrease in the extent of extreme and very extreme droughts, although severe droughts still predominated in some regions. This fact shows an indication of improving hydrometeorological conditions but also confirms that the risk of drought remains an ongoing issue for the agricultural sector in West Sumatra. Spatially, drought vulnerability appears consistent across the West Pasaman, South Pesisir, and South Solok Regencies. These three regions have extensive rice paddy fields, but most still rely on rain-fed irrigation systems, making them highly vulnerable to seasonal rainfall declines. In contrast, Solok City shows a significant reduction in the area affected by extreme drought, which can be attributed to the relatively better availability of irrigation infrastructure, smaller area coverage, and more efficient water resource management. These findings indicate that differences in drought vulnerability across regions are determined not only by climate variability but also by local adaptive capacity, the availability of irrigation infrastructure, and the effectiveness of water resource management.

The spatial discrepancy between SPI and NDDI can also be explained by differences in topography, irrigation systems, land management, and rice crop phenology. Topographic conditions influence surface runoff, drainage, water accumulation, and soil moisture retention. Areas with relatively flat terrain may retain water for longer periods, whereas areas with higher slopes tend to experience greater runoff and faster drainage, potentially increasing agricultural water stress even when rainfall conditions are relatively normal. In addition, differences in irrigation infrastructure and water distribution capacity may cause unequal water availability among rice fields. Irrigated areas with adequate and well-maintained irrigation networks are generally more capable of maintaining crop water availability during short-term rainfall deficits, whereas rain-fed or poorly served areas are more vulnerable to water stress.

Land management practices may further modify the response of rice fields to rainfall variability. Practices such as water management, soil moisture conservation, field drainage, organic matter management, and cropping patterns can affect the amount of water retained and available to crops. Consequently, two areas receiving similar rainfall may exhibit different vegetation and surface moisture conditions as detected by NDDI. Crop phenology is also an important consideration because rice water requirements and vegetation responses vary among growth stages. During the vegetative and reproductive stages, increasing canopy development and crop water requirements may result in stronger responses to water deficits. Therefore, differences in planting dates and crop growth stages among rice fields can contribute to spatial variations in NDDI values. These factors demonstrate that agricultural drought is a multidimensional phenomenon in which rainfall deficits interact with local physical conditions, irrigation availability, land management, and crop development. Thus, the integration of meteorological and satellite-based indicators provides a more comprehensive assessment of drought conditions in rice fields than relying on rainfall-based indicators alone.

CONCLUSION

West Sumatra Province experienced periods of drought, which tended to occur in July between 1994 and 2023. Analysis using the Standardised Precipitation Index (SPI) recorded 370 drought events during this period. In July 2023, the area of rice fields affected by drought, based on the SPI, was categorised as dry and somewhat dry, with a total area of 2,377.40 ha, or 1.01% of the total rice field area in West Sumatra Province. The most severely affected area, classified as somewhat dry, was Pasaman Regency, with an area of 2,030.39 ha. Results of the drought analysis using the Normalised Difference Drought Index (NDDI) showed that the extent of the drought was categorised as severe, extreme, and very extreme, with a total area of 198,772.11 ha, or 84.76% of the total rice field area in West Sumatra Province. The most severely affected area, classified as very extreme, was Padang Pariaman Regency, with an impact area of 8,799.41 ha.

The integration of SPI and NDDI provides complementary information for drought assessment in rice fields in West Sumatra. SPI characterises rainfall anomalies at the meteorological scale, whereas NDDI captures spatial variations in vegetation and surface moisture stress. The resulting drought information can support drought management by helping identify priority areas for irrigation allocation, water conservation, irrigation-network rehabilitation, and adaptive crop management. However, because independent field-scale drought observations were limited, the NDDI results should be considered a spatial assessment rather than a fully validated agricultural drought product. Further research should incorporate soil-moisture observations, agricultural drought records, and crop productivity data to strengthen quantitative validation.

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