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Pigeon Pea (*Cajanus cajan*) Leaf Flavonoid Production at Different Cow Manure Rate Application and Pruning Height

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ABSTRACT

Pigeon pea (*Cajanus cajan*) seeds are widely consumed as a staple food in numerous countries, with leaves recognized for various medicinal values, particularly in flavonoid production. Therefore, this study aimed to investigate leaf flavonoid production in pigeon peas through recurrent harvesting at different heights and the application of cow manure rate. The experiment was carried out in a Split Plot Design, with the main plot consisting of three levels of cow manure at 0, 15, and 30 tons/ha, while the subplots comprised no pruning, 100 and 125 cm above the ground. The findings indicated no observed interaction between cow manure and pruning concerning the variables studied. Recurrent harvesting with 30 tons of cow manure/ha produced a total leaf flavonoid of 2228.3 mg quercetin equivalent (QUE)/100 g leaf dry weight. It showed that 30 tons of cow manure/ha needed to be reapplied for three consecutive harvests since the value declined from the second to the third harvesting. Pruning at different heights above ground produced no significant differences in total leaf flavonoid per plant, with a range of 841.10–1,539.00 mg QUE/100 g leaf dry weight.

Keywords: Manure reapplication, medicinal plant, recurrent harvesting, quercetin

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INTRODUCTION

The pigeon pea (*Cajanus cajan*) is a flexible legume that has served as a dietary staple

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in diverse cultures for centuries. This legume is a significant protein source in numerous tropical and subtropical regions, including India, Africa (Fuller et al., 2019), and Indonesia. Primarily grown as seeds, the other parts of pigeon peas, such as leaf, can be used as a medicinal plant (Aja, Igwenyi et al., 2015; Fuller et al., 2019). Based on comparative analysis, the leaf of pigeon peas contains higher concentrations of bioactive compounds than the seeds. Specifically, the levels of flavonoids were found to be 423.75 ± 57.81 and 31.08 ± 8.20 mg/100g in leaf and seeds, respectively. Tannins were 31.55 ± 2.67 and 17.30 ± 0.47 mg/100g, alkaloids had values of 3118.86 ± 79.35 and 385.54 ± 75.15 mg/100g, saponins were 51.21 ± 4.66 and 1.82 ± 0.29 mg/100g, cyanogenic glycosides were 43.91 ± 5.99 and 12.42 ± 1.84 mg/100g, glycosides were 3.55 ± 1.98 and 3.80 ± 1.01 mg/100g, and anthocyanins were 8.35 ± 0.172 and 4.75 ± 0.174 mg/100g in leaf and seeds, respectively (Aja, Alum et al., 2015). A previous study reported that repeated doses of a combination of pigeon pea leaf extract and ginger rhizome showed no toxic effect on rats (Wresdiyati et al., 2023). Various solvent extracts obtained from different parts of pigeon peas, including leaves, roots, stems, and seeds, have also been assessed for phytochemical composition and biological activities. Flavonoid evaluations as a therapeutic agent included antioxidant, antimicrobial, antidiabetic, neuroprotective, and anti-inflammatory effects, showing the medicinal properties and therapeutic potential of plants (Fuller et al., 2019; Gargi et al., 2022; Oke, 2014; Ullah et al., 2020).

Pigeon peas thrive in warm and semi-arid conditions, showing their suitability for tropical and subtropical climates. It grows optimally in areas with temperatures ranging from 20°C to 30°C (68°F to 86°F) and requires well-drained soil (Abebe, 2022). Although pigeon peas can endure different soil types, optimal performance is mostly observed in sandy loam or loamy soils within a pH range of 6.0 to 7.0. The potential to withstand dry periods makes pigeon peas suitable for regions with irregular rainfall (Musokwa & Mafongoya, 2021). Additionally, pigeon peas can fix atmospheric nitrogen through symbiotic relationships with certain bacteria (Fossou et al., 2016).

Fertilization practices for pigeon peas should be focused on soil characteristics and specific regional requirements, particularly the Nitrogen Phosphorus Potassium (NPK) fertilizer (Ahmed et al., 2021). Typically, phosphorus shows a positive response from pigeon peas, which require enough calcium, potash, and magnesium for proper growth and development (Pal et al., 2011). Moreover, the balanced use of fertilizers, adding organic matter and nitrogen-fixing ability contribute to this versatile legume's productive and environmentally friendly cultivation. Mago and Bunga (2020) stated that there is no significant increase in pigeon pea seed productivity with the application of 2.5 kg cow manure m^{-2}, equivalent to 25 tons per ha.

Nutrient levels play a crucial role in determining the yield of pigeon pea cultivars. Based on previous studies, applying 45 kg N/ha of urea significantly enhanced the dry matter of pods and grain yield. The combination of urea at 45 kg N/ha with 10 tons of manure/ha showed positive effects on the growth parameters, including stems and branches, pods dry matter, as well as the number of primary branches. Additionally, applying 120 kg P205/ha in the form of TSP contributed to increased grain yield. Plants grown during short rains also showed greater height compared to those cultivated in long rains (Mukindia, 1992). In sandy loam soil during summer plantations, the fertilization of pigeon pea plants with 25 m^3/fed of cow manure resulted in a significant increase in fresh green forage for animals, reaching 21.55 tons/fed. Subsequent fertilization with mineral nitrogen at a rate of 80 kg/fed also contributed to enhanced forage production (El-Seifi et al., 2013).

Pigeon pea leaf has been produced on nodes, which remains unaffected by season and plant density of 4±33 plant m^{-2}. The rate of senescence of the main stem node, regarding thermal time, was also unaffected by plant density and growth duration (Ranganathan et al., 2001). In one of the references, an inorganic fertilizer consisting of 100 kg/ha with 19% nitrogen (N), 38% phosphorus pentoxide (P_2O_5), and 7% sulfur (S) was applied. Subsequently, harvesting was performed with three repetitions by cutting the plant 50 cm above the ground during the flowering stage. The results showed that plots with wider interrow spacing had higher levels of leaf crude protein and in vitro digestible organic matter compared to those with narrower row spacing (Mekonen et al., 2022). Recurrent harvesting with different cutting intervals affected vegetative growth variables and forage yield (Abidinsyah et al., 2020), and the cutting height of 50 cm above the ground with 4 weeks harvesting interval produced the highest fodder yield (Bode et al., 2018). Cow manure, recurrent harvesting, and cutting height are supposed to influence secondary metabolites, including flavonoids, as was found in waterleaf (*Talinum triangulare*) (Saleh et al., 2014). Flavonoids, naturally occurring compounds synthesized in various plant tissues, demonstrate significant antioxidant capabilities. They effectively regulate reactive oxygen species (ROS) buildup by scavenging them upon their formation. As a result, these antioxidant compounds play a crucial role in enhancing plant stress resilience (Dias et al., 2021). The flavonoids can be used as a bioactive marker to standardize the bioactive compounds related to medical benefits, including antioxidant and antidiabetic activities (Al-Masri et al., 2023; Ullah et al., 2020). Consequently, through organic cultivation, this study aimed to determine the best fertilizer rate and pruning height for leaf and flavonoid production in pigeon peas.

MATERIALS AND METHODS

This study was carried out at the IPB Biopharmaceutical Cultivation Conservation Unit Research Station, Bogor, Indonesia, from March to December 2023. The materials used included semi-determinate pigeon peas from Lombok, West Nusa Tenggara, Indonesia, and cow manure. The experiment was conducted using a Split Plot Design, with the main plot having three levels: cow manure 0, 15, and 30 tons/ha, while the subplots included no pruning, 100 and 125 cm above the soil. Each experimental unit comprised 8 plants, and the entire setup included three replications, resulting in 216 plants.

Cow manure analysis showed a pH of 7.41, containing 37.41% C-organic, N-total of 1.90%, P_2O_5 1.50%, K_2O 2.13 mg/100g, Ca^{2+} 1.52%, and Mg^{2+} 0.54%. The soil analysis showed that the pH was 4.44 (low), containing 2.22% C-organic (medium), N-total 0.27% (medium), P 3.54 ppm (very low), Ca 3.87 cmol $^{(+)}$/kg (low), Mg 1.18 cmol $^{(+)}$/kg (medium), K 0.15 cmol $^{(+)}$/kg (low), and cation exchange capacity of 24.43 cmol $^{(+)}$/kg (medium).

In this study, one and half months old seedlings were transplanted to the field. Initially, land clearing was carried out by cleaning weeds, followed by planting holes, and the application of cow manure according to the treatment rate, the whole rate at one time, with a spacing of 1 m x 1.5 m. Harvesting was performed by cutting the shoot or above-ground part of the plant-based on specified treatment. Observations were made monthly on production components in leaf number, plant height, branch number, relative growth rate (RGR), and net assimilation rate (NAR). According to the experimental procedure, RGR and NAR for the treatment without pruning would be carried out every month for 6 months, while for pruning height treatment of 100 and 125 cm, sampling for RGR and NAR was performed until flowering time (the RGR and NAR computed in days). Leaf harvesting was carried out when 50% of the population of plants had started to flower at a height of 30 cm from the initial pruning height (3 months after planting; MAP). Leaf harvesting is executed by pruning the plant according to the pruning height. The leaf was weighted and expressed as plant weight. At the second harvest (5 MAP), leaf NPK was analyzed with N using the Kjehldahl method, while P and K applied the fresh ashing method with a mixture of Nitric Acid and Perchloric Acid. Furthermore, with modification, flavonoid analysis was conducted using a spectrophotometer UV-VIS Shimadzu UV-1280 (Japan), according to Vongsak et al. (2013). The modification executed is the reduction in absorbance resulting from the reaction reduced by the absorbance of the sample that was not reacted with Aluminum Chloride. The addition of potassium acetate was adjusted according to the method of Chang et al. (2002) to reduce turbidity due to reaction with $AlCl_3$. The flavonoid concentration is multiplied by the leaf dry weight to find the flavonoid produced per plant. The third harvest was carried out at 7 MAP, and the data obtained were subjected to analysis of variance. The Duncan Multiple Range Test followed it with a significant level of α 5% for further analysis and comparisons.

$$\text{Relative growth rate /RGR} = (\ln W2 - \ln W1)/\,(t2 - t1) \qquad [1]$$

Remarks: $W1$ and $W2$ = plant dry weights at times $t1$ and $t2$; t=months

$$\text{Net assimilation rate /NAR} = (W2 - W1)/\,(t2 - t1) \times (\ln LA2 - \ln LA1)/\,(LA2 - LA1) \qquad [2]$$

Remarks: $W1$ and $W2$ = plant dry weights at times $t1$ and $t2$
$LA1$ and $LA2$ = leaf area at times $t1$ and $t2$

RESULTS AND DISCUSSION

No interaction between cow manure and pruning affected all the variables observed. Table 1 shows the climatic conditions during the experiment, indicating that the drought season in Bogor usually lasts from May/June to September. Except for September 2023 (87.3 mm/month), rainfall intensity in all months is considered wet or rainy season, although the temperature is relatively high. With enough rainfall, these conditions significantly enhanced pigeon peas' vegetative growth.

Table 1
Climatic conditions during the growing season of March–October 2023

	Mar	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
T (°C)	25.7	26.7	27.0	26.5	26.3	26.3	26.8	27.6
Tmax (°C)	31.3	32.6	32.9	32.5	32.3	33.1	33.7	34.6
Tmin (°C)	22.2	22.9	23.5	23.1	22.4	21.9	21.5	23.1
RF (mm)	325.8	312.8	294.2	310.7	134.4	134.4	87.3	180.7
RH (%)	86.1	83.7	82.4	83.8	79.5	76.0	71.8	74.4
SD (h)	4.9	6.1	6.2	7.1	6.9	7.8	8.1	7.7

Note. T=Average temperature; Tmax=Temperature maximal; Tmin=Temperature minimum; RF=Rainfall; RH=Relative humidity; SD=Sunshine duration (Indonesia Meteorology, Climatology, and Geophysical Agency, 2023)

A previous study in Gunung Kidul, Yogyakarta, Indonesia, with less optimal soil conditions and low rainfall intensity, showed the survival of 30 black pigeon pea types (Yuniastuti et al., 2020). However, this study was conducted in Bogor, which is characterized by a better soil analysis that contributes to plant growth. The soil analysis showed a low pH of 4.44, containing 2.22% C-organic (medium), N-total of 0.27% (medium), P 3.54 ppm (very low), Ca 3.87 cmol $^{(+)}$/kg (low), Mg 1.18 cmol $^{(+)}$/kg (medium), K 0.15 cmol $^{(+)}$/kg (low), and cation exchange capacity of 24.43 cmol $^{(+)}$/kg (medium).

In plants without pruning, the application of manure to pigeon peas did not affect the RGR and NAR from 1 to 6 MAP. The significant fast growth rate, as shown by the RGR in Figure 1 and NAR in Figure 2, was observed during the initial 1 to 2 MAP, followed by a decline to 6 MAP. These semi-determinate plants elongated with higher height produced branches with leaves on the nodes, which started flowering at 3 MAP and produced pods with more branches. The flowering reduced the vegetative growth rate due to the movement of the sink from leaf buds to flowers or pods. The results showed that different growth stages have various photosynthate translocations, with the blooming stage being lower (Isobe et al., 2020). In contrast, pruning or harvesting in this experiment removed the flowers, causing increased biomass production, as observed in *Hesperaloe funifera* (Agavaceae) (McLaughlin, 2003). The integrative developmental stages identified in mango included growth asynchronisms between two topologically connected organs: the vegetative axis and leaf. This phenomenon was explained by examining the coordinated development between the vegetative axis and leaf during various stages of growth (Dambreville et al., 2015).

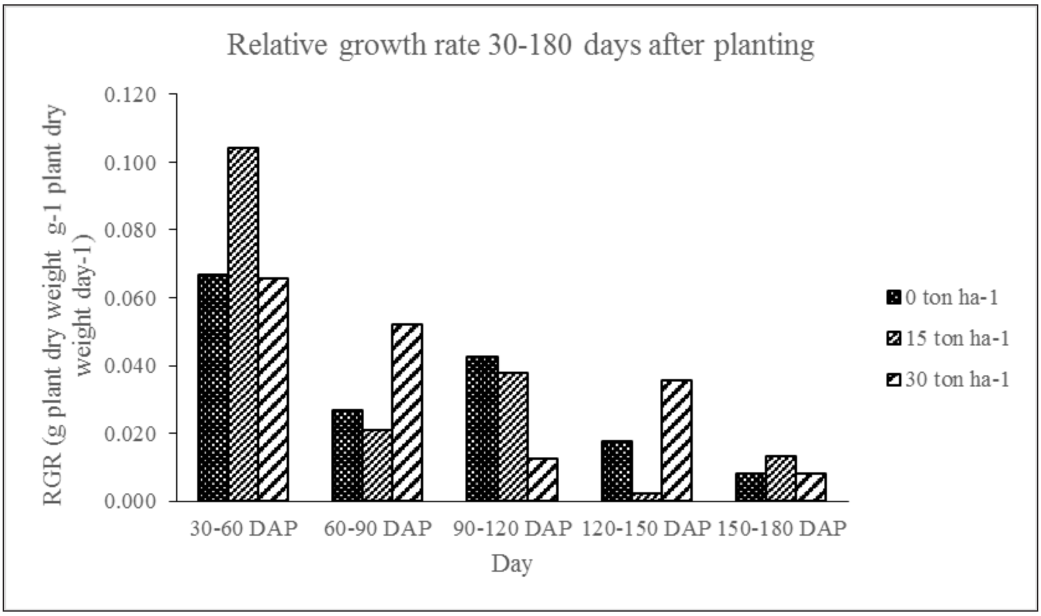

Figure 1. Relative growth rate 30–180 days after planting (DAP) with different cow manure rate

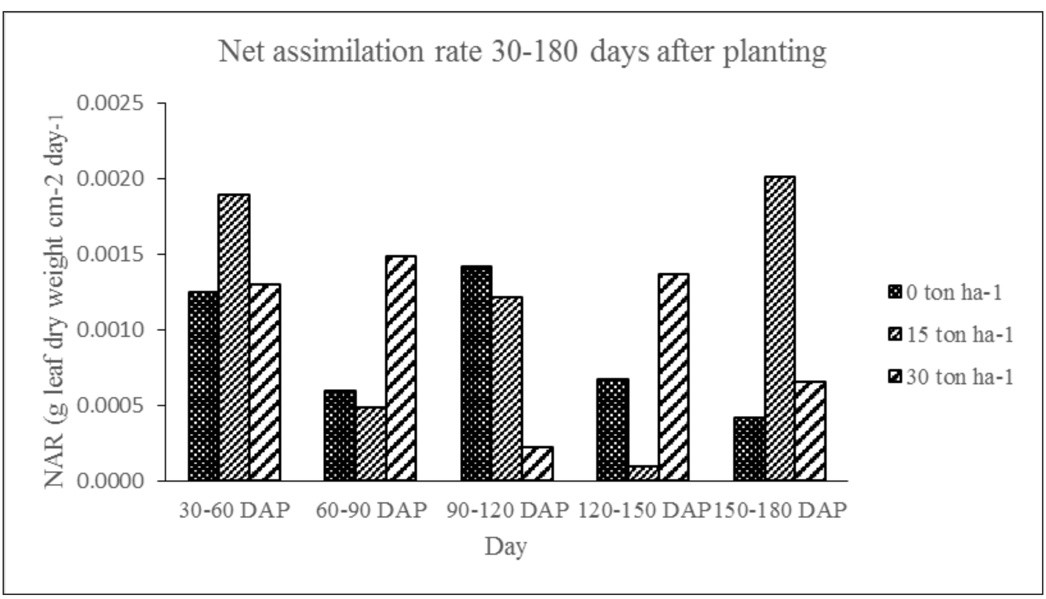

Figure 2. Net assimilation rate 30–180 DAP with different cow manure rate

Plant heights ranging from 1 to 6 MAP remained unaffected by the application of cow manure. Nonetheless, notable disparities in plant height were observed at 5 MAP following two rounds of harvesting at 3 and 5 MAP, particularly evident in Table 2. Based on observation, the data showed that pruning at 100 and 125 above ground produced significantly lower heights of 53.22 and 44.15%, respectively, compared to 6 MAP. Before the first harvesting, approximately 3 MAP 30 tons of cow manure/ha had 145.81 and 39.39% significantly higher leaf numbers than without or 15 tons of cow manure/ha, respectively. At 4 MAP, 30 tons of manure/ha produced 83.02% significantly higher leaf numbers than without manure, as shown in Table 3.

Table 2
Plant height 1–6 Months After Planting (MAP) with different cow manure rates or pruning heights

Treatment	Plant Height					
	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP
	……………………………………cm……………………………………					
Cow manure rate						
0 ton ha^{-1}	86.20	150.01	202.30	189.04	235.36	188.45
15 ton ha^{-1}	74.83	147.36	208.86	186.2	219.11	180.08
30 ton ha^{-1}	85.11	158.12	212.82	207.61	225.74	182.48
Pruning height (cm)						
0	82.71	153.64	215.96	237.8	262.37a	271.94a
100	78.47	148.11	198.59	174.71	199.54b	127.20c
125	84.96	153.74	209.42	170.33	218.51b	151.87b

Note. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test

Table 3
Leaf number 1–6 Months After Planting (MAP) with different cow manure rates or pruning height

Treatment	Average leaf number					
	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP[1)]	6 MAP[1)]
Cow manure rate						
0 ton ha^{-1}	29.2	154.5c	352.4b	264.4b	444.2	235.7
15 ton ha^{-1}	24.0	190.2b	466.2b	375.6ab	462.5	239.8
30 ton ha^{-1}	32.3	259.6a	649.8a	483.8a	549.5	222.6
Pruning height (cm)						
0	30.0	202.0	516.6	667.6a	602.2a	368.0a
100	27.1	192.4	464.2	156.3c	316.7b	110.3c
125	29.1	209.8	487.6	299.9b	537.3a	219.8b

Note. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test; [1)] =Transformation $\sqrt{x+0.5}$

The growth of pigeon peas for leaf harvesting follows a sequential condition influenced by plant height due to node growth from the stems and branches. According to Ranganathan et al. (2001), longer internodes would reduce node number, while higher pruning height produced lower leaf number. In this study, pruning caused leaf number differences from 4 to 6 MAP. A significantly higher leaf number was found at 6 MAP on plants without pruning, 100, and 125 cm pruning above the ground, as shown in Table 3. Plants without pruning produced significantly higher leaf numbers of 70.02 and 40.27% compared to 100 and 125 cm above ground, respectively.

In this study, 30 tons of manure per hectare influenced branch number compared to the control at 2, 3, and 5 MAP, with a significant increase of 40.74, 43.35, and 115.09%, respectively. Although harvesting at 3 MAP produced a similar branch number at 4 MAP, higher manure rates at 5 MAP were more significant compared to the control, as shown in Table 4. The results also showed that branch numbers were affected by cow manure after 2 months of harvesting. This phenomenon suggested a time lag in nutrient usage due to the slow release (Prado et al., 2022; Saputra et al., 2019) of cow manure (Prado et al., 2022).

Table 4

Branch numbers 1–6 Months After Planting (MAP) with different cow manure rates or pruning height

	Average branch number					
Treatment	**1 MAP**	**2 MAP**	**3 MAP**	**4 MAP[1)]**	**5 MAP[1)]**	**6 MAP[1)]**
Cow manure rate						
0 ton ha^{-1}	4.3	10.8b	14.3b	24.3	26.5b	29.0
15 ton ha^{-1}	4.6	14.4a	18.2ab	32.0	42.7ab	37.3
30 ton ha^{-1}	5.9	15.2a	20.5a	42.5	57.0a	35.6
Pruning height (cm)						
0	5.0	14.0	18.4	17.5b	17.5c	17.5c
100	4.8	12.4	16.4	29.3b	41.8b	30.6b
125	5.1	14.0	18.2	52.0a	66.9a	53.8a

Note. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test; [1)] =Transformation $\sqrt{x+0.5}$

After the first harvest at 3 MAP, pruning height significantly produced higher branch numbers at 4, 5, and 6 MAP. Branch numbers for pruning height of 100 cm and 125 cm at 6 MAP, which recorded an increase of 74.86% and 207.43%, were significantly higher compared to the control. Furthermore, recurrent harvesting of pigeon peas showed affected plant height, leaf, and branch number (Tenakwa et al., 2022).

Leaf increased with manure application, including fresh stem and dry weight, as shown in Figures 3A and 3B. In the first harvest, the application of 30 tons of cow manure/ha produced leaf fresh and dry weight, which was significantly higher at 125.74 and 174.94%

compared to those without manure. Furthermore, the total leaf freshness of 554.59 g/plant and the dry weight of 204.24 g/plant were significantly higher than the control, 108.93 and 115.68%, respectively. Plants with and without cow manure had the highest leaf dry weight in the second harvest, which significantly declined in the third harvest. It showed that after the second harvest, the nutrients from cow manure decreased, indicating the need for reapplication.

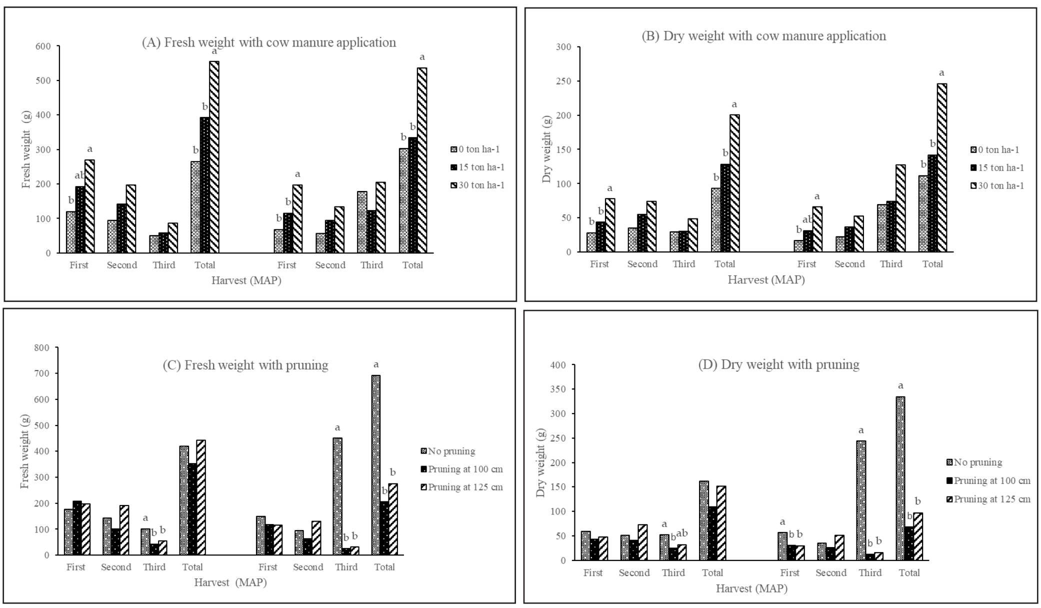

Figure 3. Fresh (A) and dry weight (B) with manure application; fresh (C) and dry weight (D) with pruning. Bars with different letters in the same harvesting time above them are significantly different in the 5% DMRT test

Total stem fresh (536.55 g/plant) and dry weight (245.82 g/plant) with 30 tons of cow manure/ha was significantly higher than control at 77.10 and 119.93%, respectively. During the second harvest, the application of cow manure and pruning height did not affect the leaf weight, including stem fresh and dry weight. At the third harvest, no significant increase was observed in the fresh and dry weight of the leaf and stem. However, it was observed that recurrent harvesting led to an increase in the fresh and dry weight of the stem.

Plants without pruning produced significantly higher leaves and stem fresh weight at third and total harvest, as shown in Figure 3C. The absence of pruning resulted in significantly higher leaf dry weight during the third harvest. Additionally, stem dry weight was significantly higher on the first, third, and total harvest when no pruning was implemented, as shown in Figure 3D. This phenomenon could be accepted, as harvesting at a certain height reduced harvest weight. Tenakwa et al. (2022) stated that the cutting regime for pigeon peas significantly affected the biomass yield, while further cutting at 20 weeks after planting (WAP) produced more biomass compared to 12 WAP.

The results also showed that leaf and stem weight decreased from the first to the second harvest, while a significant increase was observed at the third harvest, contributing to total stem weight. Without pruning, plants in the generative phase produced more stems than leaves. Furthermore, harvesting at 100 cm above ground produced insignificant lower fresh and dry leaves compared to 125 cm above ground. The presence of more branches due to pruning contributed to harvesting younger leaves with higher water content.

The application of cow manure significantly increased leaf N and P, as added fertilizers met the crop's nutrient requirement. However, no substantial effect was observed on leaf K, as shown in Table 5. The results showed that cow manure supplied 37.41% C-organic, N-total 1.90%, P_2O_5 1.50%, K_2O 2.13%, Ca^{2+} 1.52%, and Mg^{2+} 0.54%. Leaf N with the application of 15 and 30 tons of manure/ha was 7.12 and 9.87%, significantly higher than control. Furthermore, leaf P with 30 tons of cow manure/ha was 14.29% significantly higher compared to without manure. In soybean leaf, nutrient sufficiency for N, P, K, Ca, and Mg was 3.86–4.57%, 0.31–0.37%, 1.83–2.07%, 0.94–1.13%, and Mg 0.44–0.53% (Souza et al., 2020). Based on soybean reference, pigeon pea leaf N was sufficient with 30 tons of cow manure/ha application, while P and K were insufficient. It showed that the application of cow manure at 30 t/ha could not supply the P needed by pigeon peas, playing a fundamental role in regulating abiotic stress tolerance (Khan et al., 2023). Despite the low nutrients found in cow manure (Prado et al., 2022), N, P, and K content contributed to the growth and leaf harvest in pigeon peas. Adding organic matter also contributes to long-term nutrient availability and soil health, which may require fertilization (Goldan et al., 2023). In mung beans, N, P, and K presence facilitated root development, flowering, and pod formation (Yin et al., 2018). On other species, organic matter increased the growth variables, namely plant height, branch number, leaf freshness, and dry weight, such as in *Vernonia amygdalina* (Tjhia et al., 2018).

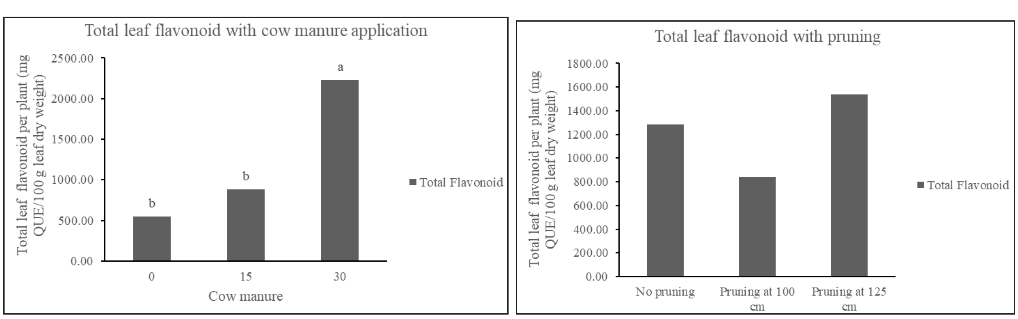

Figure 4. Total leaf flavonoid per plant from 3 times harvesting with cow manure application (left) and pruning height (right). Bars with different letters above them are significantly different in the 5% DMRT test

Table 5
Leaf NPK content at 5 Months After Planting (MAP) and leaf total flavonoid per plant for three harvesting with different manure rates or pruning height

Treatment	**N**	**P**	**K**	**Leaf total flavonoid**
	………………% dry weight……………			mg QUE/100 g leaf dry weight
Manure rate				
0 ton ha^{-1}	3.65b	0.21 b	0.80	523.3b
15 ton ha^{-1}	3.91a	0.22ab	0.78	618.9b
30 ton ha^{-1}	4.05a	0.24 a	0.86	993.5a
Pruning height (cm)				
0	3.73	0.22	0.73	725.3
100	3.96	0.23	0.90	630.4
125	3.92	0.21	0.81	780.0

Note. Numbers followed by different letters in the same column indicate significantly different results in the 5% DMRT test; QUE = quercetin equivalent

Total leaf flavonoid production per plant from three times harvesting with application of cow manure and pruning height was shown in Figure 4. The application of 30 tons of cow manure per hectare showed a significantly higher value compared to both without manure and 15 tons, accounting for an increase of 115.68 and 55.88%, respectively. Higher cow manure dosage contributed to greater nutrients available for plants. Adding 25 tons of cow manure per ha does not increase pigeon pea seed production in India (Mago & Bunga, 2020), but no data was found for fodder production. The Latosol soil in Indonesia has low organic matter (Adhi et al., 2017; Suminar et al., 2017), which shows the need for manure application. A minimum of 5 t cow manure/ha should be applied (Hatibie & Garantjang, 2022). Leaf total flavonoids for 30 tons of cow manure/ha application also showed a significantly higher value, as presented in Table 5. In *Centella asiatica*, repeated harvesting with organic manure showed higher phytoconstituents, such as flavonoids (Bhattacharya et al., 2017).

This value could be explained by the significantly higher leaf N and P. Specifically, N as part of the chlorophyll molecular formula contributed to the photosynthetic processes that produced plant growth and biomass (Ebrahimi et al., 2023). P plays a crucial role in energy transfers, photosynthesis, as shown by Kayoumu et al. (2023), nutrient flow, and plant growth (Khan et al., 2023). In this study, the analysis of the dry leaf harvesting from Figures 1A and B showed that for the third harvesting, the application of 30 tons of cow manure/ha was insufficient, indicating the need for manure reapplication. Additionally, pruning with different heights above ground produced no significant differences in total flavonoid production in the leaves per plant.

CONCLUSION

This study showed that leaf flavonoid production in pigeon peas using 30 tons of cow manure/ha resulted in 2228.3 mg QUE/plant. The results showed the need for reapplication of 30 tons of cow manure/ha three times harvesting, as the value declined from second to third harvesting. Additionally, pruning at various heights above the ground did not result in significant differences in leaf total flavonoid production per plant, with values ranging from 841.10 to 1,539.00 mg QUE/plant.

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