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Bioefficacy of Bio-insecticide from *Chromolaena odorata* (L.) R. M. King & H. E. Robins Methanol Extract against Brown Planthopper, *Nilaparvata lugens* (Stål.)

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ABSTRACT

The rice brown planthopper (BPH), *Nilaparvata lugens* (Stål.), is a highly damaging insect pest to rice crops. The excessive use of synthetic chemicals has resulted in the development of resistance to insecticides and negative consequences for the environment and insect biodiversity. Hence, three common weed species, namely *Ageratum conyzoides*, *Chromolaena odorata*, and *Mallotus paniculatus*, were evaluated on the comparative extraction yield in different solvents, as well as the toxicity potential in the selected methanol extract. Further, the bioactive compounds in *C. odorata* were characterized, and potential bio-insecticide formulations were developed and evaluated on the BPH. Methanol extract displayed higher efficiency, yielding 17.29% compared to only 3.19% in hexane extract. Insecticidal activity evaluation demonstrated that *C. odorata* exhibited the highest toxicity (77.50% at 10,000 ppm), having a median lethal concentration (LC_{50}) value of 977 ppm, while *A. conyzoides* (55.20% at 10,000 ppm) and *M. paniculatus* (60.0% at 12,000 ppm) produced LC_{50} values of 6,549 ppm and 21,940 ppm, respectively. Subsequently, a plant-based bio-insecticide was formulated using the crude methanol extract of *C. odorata* as the active ingredient. A mixture of surfactant (Emersense® AM 8025), oil (palm kernel oil ester), and water resulted in a stable macroemulsion called Emersense® AM 8025/palm kernel oil

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ester/water (EM-PKOE). The formulated macroemulsion displayed enhanced toxicity and efficacy against BPH nymphs, with an LC_{50} value of 220 ppm, outperforming the unformulated crude methanol extract (977 ppm). Chemical composition analysis using gas chromatography-mass spectrometry revealed that *C. odorata* primarily contained sesquiterpenes (24.14%). This study proposes *C. odorata* as a potential bio-insecticide for BPH combatants, necessitating further research on the formulation for eventual commercialization to sustainable BPH control in rice cultivation.

Keywords: Bio-insecticide, brown planthopper, crude plant extract, emulsion formulation, gas chromatography-mass spectrometry

INTRODUCTION

Brown planthopper (BPH) (*Nilaparvata lugens* (Stål).; Hemiptera: Delphacidae) has always been a major threat to rice crops globally, causing 20–80% yield loss (Balachiranjeevi et al., 2019). Heavy infestation of BPH results in a dried-out rice stand phenomenon called 'hopperburn'. Locally known as 'bena perang'. BPH is also recognized as a vector of rice grassy stunt virus (RGSV) and rice ragged stunt virus (RRSV) (Jena & Kim, 2010). In late 2017, the Muda Agricultural Development Authority (MADA) granary experienced tremendous BPH infestation, involving a vast 800 ha (Khazanah Research Institute, 2019), followed by 213.70 ha in 2021 with 76.40 ha of hopperburn cases reported (Hashim, 2021). Similarly, in the Integrated Agricultural Development Area (IADA) Rompin rice granary, Pahang, BPH infestation had caused an extreme decline in rice production to as low as 2–3 t/ha (Khazanah Research Institute, 2019). Meanwhile, in Bagan Serai, Perak, about 79 ha of rice fields were damaged by BPH in January 2021, causing an RM24,000 loss to rice growers involved in the incident (Pauzi, 2021). In the following year, a group of 1,000 farmers in Yan, Kedah, incurred more than 50% yield losses after their crops were attacked by BPH (Awang, 2022). Meanwhile, in Pokok Sena, a total of 17 ha of rice fields were also afflicted by BPH, resulting in losses of nearly RM60,000 (Mansor, 2022).

Currently, farmers opt for synthetic insecticides to control BPH. Inevitably, intensive and excessive use of synthetic insecticides has caused several deleterious effects on human health, environmental degradation, and insect resurgence and resistance (Matsumura & Sanada-Morimura, 2010). Among the synthetic insecticides that have yielded resistant BPH populations are imidacloprid (Garrood et al., 2016), neonicotinoids carbamates, organophosphates, cyclodiene organochlorines, phenylpyrazoles (fiproles), pyrethroids-pyrethrins, and buprofezin (Khoa et al., 2018). Alternatively, a more sustainable BPH control method that renders less harmful environmental effects should be emphasized. Botanical pesticides, which are more environmentally benign, are currently being considered.

Various plant species, particularly medicinal plants, have numerously been

reported to possess insecticidal properties such as nicotine, rotenone, and pyrethrum (Said-Al Ahl et al., 2017; Stevenson et al., 2014). Nonetheless, weeds that are regarded as unwanted plants have always been understudied. Weeds are rather recognized as a nuisance, strong competitors, highly persistent, well adaptable to changing climate, and, to a certain extent, capable of exerting depressive effect upon the adjacent vegetations via allelopathic interactions. Equally, these special properties allow weeds to deter some insects and plant diseases, making them among the successful survivors in natural or disturbed ecosystems. Therefore, weeds could offer great potential as plant-based biopesticides as they are relatively biodegradable and can become a crucial alternative to harmful synthetic chemicals.

In Malaysia, *Ageratum conyzoides* (L.), *Chromolaena odorata* (L.) R. M. King & H. E. Robins, and *Mallotus paniculatus* (Lam.) Mull. Arg. are common weeds in agricultural ecosystems. *Ageratum conyzoides* plant extracts effectively controlled cowpea weevil (Gbolade & Adebayo, 1993) and grasshopper (Ingrid et al., 2020). Whereas *C. odorata* successfully caused mortality in rice weevils (Acero, 2014) and blackflies (Matur & Davou, 2007). In addition, *M. paniculatus* and other Euphorbiaceae were also reported to have insecticidal properties against *Plutella xylostella* L. (Uma & Kumar, 2009). The cause of insect mortality from these weed species was due to bioactive compounds such as saponin, alkaloids, flavonoids, terpenoids, and tannins (Udebuani et al., 2015). Nonetheless, information regarding the insecticidal activities of these three weed species against BPH is still lacking.

Plant bioactive compounds could be extracted by conventional or non-conventional methods (Azwanida, 2015). A very common method used to extract plant secondary metabolites is solid-liquid solvent extraction, also known as Normal Soaking Extraction (NSE) or maceration with the use of organic solvent, i.e., methanol and hexane (Tiwari et al., 2011; Zhang et al., 2018). These similar polarities extraction concepts contain a complex mixture of plant metabolites, which can later be identified by chemical analysis. Gas chromatography-mass spectrometry (GC-MS) is one of the instruments used to quantify the presence of the bioactive compounds in the plant extract. GC separates many volatile and semi-volatile compounds but does not selectively detect them, while MS is capable of selectively detecting many compounds but not always separating them (Sneddon et al., 2007).

Insect mortality bioassay is advantageous when specific compounds from the plant extract are recognized for their detrimental potential. Additionally, this practice offers valuable insights, especially when conducting insecticide formulation. The effectiveness of plant extract against pests could be improved by developing a formulation of bio-insecticide prior to commercialization into the market. A pesticide formulation is a chemical mixture comprising inert materials and active

ingredients (a.i.) that effectively controls the target pests (Hazra & Purkait, 2019). Solvents, carriers, and adjuvants/surfactants are the common inert ingredients that are intentionally added into the formulation to aid the pesticide stability, handling, safety, and ease of application and increase pesticide retention-absorption capacity (Hazra & Purkait, 2019). An active ingredient is a substance that prevents, kills, or repels a pest. The introduction of inert ingredients may aid in applying the active ingredient (National Pesticide Information Center, n.d.). Emulsion formulation is an example of a pesticide formulation. It can be macro, micro, or nano-emulsion, oil-in-water, or water-in-oil. The characteristics of the emulsion are based on the ratio of the components during the emulsion preparation and the compatibility of the emulsion with the active ingredients.

Thus, the present study explores the insecticidal potential of the selected weed species in solvents having different polarities towards BPH. The bioactive compounds in the *C. odorata* methanolic extract were also characterized, and comparable bio-insecticide formulations were developed and evaluated for their bio-efficacy against BPH.

MATERIALS AND METHODS

Insect Collection and Rearing

Heong et al. (2013) adopted insect collection and rearing methods. Approximately 50 healthy and unparasitized female adults of short-winged (brachypterous) BPH (or alternatively about 100 nymphs when the number of adults was insufficient) were collected randomly using custom-made manual mouth aspirator in rice fields in Pendang, Kedah. The stock culture of BPH was maintained in a glasshouse, Ladang 10, Universiti Putra Malaysia, and had no exposure to any pesticide. The culture was maintained on MR219 rice seedlings in an individual mylar rearing cage (45 × 90 × 45 cm^3), having a day/night temperature of 33/20 ± 2°C and an average of 13 hr daylight.

Collection of Plant and Extract Preparation

Leaves of *A. conyzoides*, *C. odorata*, and *M. paniculatus* were collected from the mature plants within the Universiti Putra Malaysia. The leaves were separated and washed thoroughly under running tap water and oven-dried at 40°C (± 2°C) for 72 hr. Dried leaves were pulverized by an electric grinder (LB10S, Waring, USA). The extraction process was done by soaking the powdered sample in two solvents, which are methanol and hexane, at 1:10 w/v of solid-liquid ratio for 72 hr with constant agitation by an orbital shaker at 100 rpm. After that, the extract solutions were filtered (Whatman Nylon Membrane Filters 0.45 µm, USA), and the filtrates were concentrated by using a rotary vacuum evaporator (R-215; BUCHI, United Kingdom) at 100 rpm and 40°C. Concentrated extracts were transferred into 30 ml glass vials wrapped with aluminum foil and kept in a -20°C freezer for further use. Extraction processes were done in triplicates.

Insecticidal Activity of Selected Weeds Extracts

Preliminary contact toxicity tests were executed against the 3rd and 4th instar BPH nymphs to determine a range of concentrations of the plant extracts that cause 5–99% mortality (Nuryanti et al., 2018). A 2% crude extract concentration stock solution was prepared by adding 50 ml of methanol to 1 g of crude extract. From the stock, a series of concentrations (500, 1,000, 1,500, and 2,000 ppm) were prepared for all three weed species by adding distilled water according to the equation given below:

$$C1V1 = C2V2$$

where, C1 = Concentration of stock solution (ppm), C2 = Desired concentration to prepared (ppm), V1 = Volume from stock solution (ml), and V2 = Desired volume to prepared (ml), respectively.

From the preliminary mortality bioassay result, a new series of concentrations for *A. conyzoides* and *C. odorata* methanol extracts (100, 500, 1,000, 5,000, and 10,000 ppm, respectively) and *M. paniculatus* methanol extract (5,000, 6,000, 7,000, 8,000, 9,000, 10,000, and 12,000 ppm, respectively) were prepared as detailed above to determine the toxicity effect of the crude extracts on BPH nymphs.

A mortality bioassay was carried out using the plant dip method described in Test Method No. 005 (Nauen, n.d.). Bacteriological agar No. 1 was prepared and cooled until semi-solidified (approximately 37°C). The semi-solid agar was poured about 100 ml into each pot containing 15–21 days-old rice seedlings to cover the soil surface. After the agar solidification, the pots were reverted and dipped completely into the treatments for 10 s. Then, all the rice seedlings were allowed to dry for 60–90 min, depending on the ambient relative humidity and placed into an individual cylinder cage (22.50 cm height, 8.50 cm diameter) to prevent insects from escaping.

The 3rd and 4th instar BPH nymphs were collected from the rearing cage (nymphs cage) using a mouth aspirator. Ten nymphs were collected and left to starve for one hour. Later, all the starved nymphs were released onto the treated rice seedlings, and the cylinder cage was closed with a lid to prevent the nymph from escaping. Observation of nymphs' mortality was done at 24, 48, and 72 hr. Nymphs were considered dead if they did not respond to gentle probing with a fine sable brush. The control mortalities were corrected using Abbott's (1925) formula as below:

Corrected mortality (%)

= [% Test mortality – % Control mortality / 100 - % Control mortality] × 100%

Preparation of Emulsion Formulation

A non-ionic alkanolamide surfactant, Emersense® AM 8025 (Emery Oleochemicals (M) Sdn. Bhd., Malaysia), was incorporated in the surfactant phase of the formulation to increase the solubility of the oily component in the emulsion based on miscibility studies of the formulation. Three types of oil-based carriers, namely Edenol®

SP100 and Edenor® (Emery Oleochemicals (M) Sdn. Bhd., Malaysia) and palm kernel oil ester (PKOE) (Department of Chemistry, Faculty of Science, Universiti Putra Malaysia) were used to develop the bio-insecticide formulations. The emulsions were prepared using purified water (Elga Labwater, 18 m) by titration.

Surfactant and carrier were mixed in a 15 ml centrifuge tube at ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 9:1, and 0:10 (w/w). A non-ionic alkanolamide surfactant Emersense® AM 8025 (Emery Oleochemicals (M) Sdn. Bhd., Malaysia) was used as the surfactant, while PKOE (Department of Chemistry, Faculty of Science, Universiti Putra Malaysia), Edenol® SP100, and Edenor® (both from Emery Oleochemicals (M) Sdn. Bhd., Malaysia) were used as the oil-based carriers to develop the bio-insecticide formulations. Then, deionized water (5% [w/w]) was added by titrating into the mixtures of surfactant and carrier until 95% water content was achieved in the emulsion system.

Analytical balance (Mettler Toledo Model Dragon 204, Spain) weighed each component. The prepared compositions were homogenized using a vortex mixer (Model VTX-3000 L, Japan) and allowed to mix for approximately 3–5 min until equilibrium was achieved. The emulsions were homogenized, followed by centrifugation at 120 × *g* and 25°C for 30 min (Flanangan et al., 2006). The results obtained from the experiment were subjected to Chemix version 3.5 phase diagram plotter (United Kingdom), a software used to construct a pseudoternary phase diagram. In the pseudoternary phase diagram, several points were chosen within the isotropic region (one-phased) with the criteria of surfactant being <30% and incorporation of 5% *C. odorata* methanol leaf extract into the emulsion as the active ingredient (Table 1). The formulation that was miscible with *C. odorata* leaf extract (5%) and retained a one-phase appearance proceeded for characterization and insect bioassay.

Characterization of Emulsion Formulation

The stability test was carried out with a formulation that was miscible with *C. odorata* extract and retained the one-phase appearance. EM-PKOE emulsion incorporated with *C.*

Table 1
Ingredients used in the ternary phase diagram

Compound	Trade name	Class
Palm kernelamide DEA	Emersense® AM 8025	Surfactant
N. A.	Edenol® SP100	Carrier
Palm oil methylester	Edenor®	Carrier
Palm oil alkylester	Palm kernel oil ester	Carrier
Water	N. A.	Water
Chromolaena odorata methanol leaf extract	N. A.	Active ingredient

Note. N. A. = Not available

odorata extract was centrifuged at 120 × *g* for 30 min and kept at room temperature (25 ± 2°C) for 30 days and at 54°C for 14 days in accordance with the Food and Agricultural Organization (FAO) as a standard evaluation for agrochemical products to show the stability of the formulations in the tropical climate (Yusoff et al., 2021). The physical appearance of the emulsion was visually investigated.

Particle size, polydispersity index (PDI), and zeta potential analysis were performed on freshly prepared EM-PKOE emulsion. The formulation was diluted with deionized water in a falcon tube and gently mixed with the analyses. One (1) ml from the sample was pipetted into a quartz cell with a surface area of 1 cm^2 and placed in a Zetasizer Nano-ZS (Malvern Instruments Ltd., United Kingdom). The particle size of the formulation was determined using dynamic light scattering (DLS) to capture the Brownian motion of the dispersed phase, and the zeta potential (surface charge) of the emulsion was determined via laser doppler electrophoresis (LDE). The PDI was calculated from the photo correlation spectroscopic analysis via electrophoretic light scattering (ELS).

The viscosity test of the one-day-old formulation was measured at room temperature (25 ± 1°C) using a digital viscometer (NDJ-5S, ATO, China). Approximately 30 ml of the stock formulation was filled in the viscometer cup, followed by a rotation speed of 12 rpm at 20,000 to 100,000 millipascal-seconds (mPa.s). The measurements were taken in triplicate.

Bioassay Test for Emulsion Formulation

Mortality bioassays for emulsion formulation were conducted using the method described above with minor modifications. Five treatment concentrations (100, 500, 1,000, 5,000, and 10,000 ppm) were diluted from the EM-PKOE emulsion. Each pot containing MR219 rice plants was fully sprayed for approximately 10 s and labeled accordingly. In about an hour, bacteriological agar was filled into the pot when the treated plants were completely dried. Ten 3rd and 4th instar nymphs were released into each treated pot and encased in a plastic cylinder. Four replicates of the positive controls (Neemix 4.5® [SURECROP SDN. BHD., Malaysia] and Regent® 50SC [Bayer Co., Malaysia]) and the negative control (distilled water) were performed. The mortality of nymphs was assessed at 6, 12, 18, 24, 30, 36, 42, and 48 hr after treatment.

GC-MS

The chemical constituents of *C. odorata* methanol leaf extract were determined using the GC-MS technique (Shimadzu GC-2010 Plus, Japan), equipped with a Rxi-5ms capillary column (30.00 m × 0.25 mm inner diameter × 0.25 µm film thickness) coupled with Shimadzu GCMS-QP2010 Ultra (Japan). The oven temperature was programmed from 50 to 300°C at a rate of 3°C/min and held for 10 min. The injector temperatures were held at 250°C. The sample injected into the injector was split mode with a ratio 10. The pressure was applied at a constant flow rate of 0.80 ml/min. An electron ionization system with an

ionization voltage of 0.91 kV was used for GC-MS detection. The chemical compounds of *C. odorata* methanol leaf extract were identified by comparing their retention time and mass spectra with those recorded in databases of the National Institute of Standards and Technology (NIST) on the GC-MS system and expressed as a percentage by peak area.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA, SAS statistical software version 9.4), followed by means separation by least significant difference (LSD) test with square root transformation for extraction yields, while means separation by Tukey's multiple range test with arcsine transformation for insect mortality bioassay. The average of nymphs' mortality data was subjected to probit analysis (EPA Probit Analysis Software Program version 1.5) to calculate the LC_{50}. The values were expressed as means ± standard error of four replicates. Results with $p<0.05$ were statistically significant. The graphs were best fitted into a 4-parametric sigmoidal hill curve (SigmaPlot 14) according to the R^2 value.

RESULTS AND DISCUSSION

Extraction Yield of Plant Crude Extracts

It was observed that the extraction yields in all plant species were comparable in both solvents. Nonetheless, methanol always results in higher extraction yields in all species than hexane, as shown in Table 2.

Evidently, higher leaf extract in all selected weed species was recorded in methanol over hexane, as Prajapati et al. (2014) reported on the higher plant extract in methanol than in hexane. Solvent type, extraction method, and sample preparations are among the main factors that eventually influence the outcome of the final extracts (Azwanida, 2015). As an intermediate polar solvent, methanol also tends to extract polar compounds and some non-polar compounds (Tambellini et al., 2013). Similarly, it was also observed that high polar solvents such as methanol were more effective in extracting bioactive compounds in *Garnicia atriviridis* (Al-Mansoub et al., 2014) and *Hibiscus micranthus* (Begashaw et al., 2017) over the non-polar solvents.

Mortality Bioassay of Plant Crude Extract

In the preliminary study, the 3rd and 4th instar-nymphs of BPH were used instead

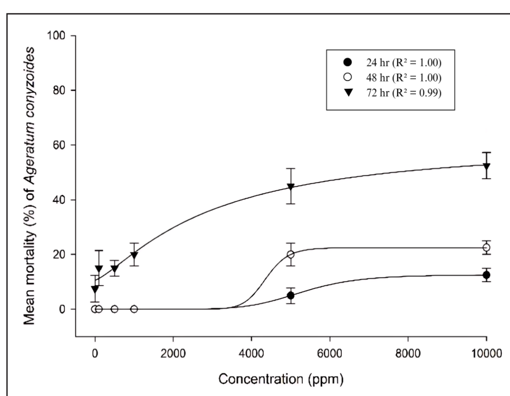
Table 2
Mean separation of extraction yield for different weed species in different solvent

Factor	*P* value
Plant	ns
Solvent	<0.0001***
Plant × Solvent	ns
Factor	**Mean of extraction yield (%)**
Plant	
Ageratum conyzoides	11.19 ± 3.61a
Chromolaena odorata	10.76 ± 3.42a
Mallotus paniculatus	8.77 ± 2.69a

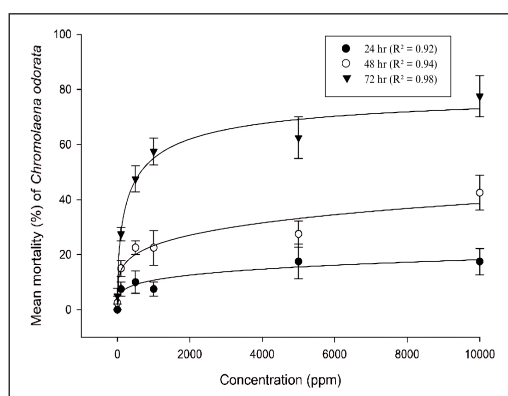
Note. *** = Highly significant, ns = Non-significant. Means followed by the same letters (among plants and solvents) are not significantly different; Least significant difference test at $p = 0.05$

of the adult because of the rapid damage caused by the nymphs compared to the adult. Saxena and Khan (1985) highlighted that when studying neem extract using different solvents (aqueous, ethanol, and hexane), conducting bioassays with early instar nymphs can provide more practical insights, especially in field experiments. Similarly, Senthil-Nathan et al. (2007) recommended using 3rd and 4th instar nymphs to target early developmental stages when conducting bioassays for BPH control, which is in line with the current study. The methanol extract of *A. conyzoides* tested against the BPH showed a highly significant mortality interaction between treatment concentration and exposure time (Figure 1). As treatment concentrations and exposure times increased, nymphs' mortality also rose. A remarkable significant in mortality was observed between the two highest concentrations (5,000 and 10,000 ppm) and the three lowest concentrations (100, 500, and 1,000 ppm). Mortality rates rose progressively from 7.50% at the control to 15% at both 100 and 500 ppm, and then to 20, 45, and 52.20% at increasing concentrations, reaching the highest level at 10,000 ppm after 72 hr of exposure. Regardless of concentration, more time was needed by the *A. conyzoides* methanol leaf extract to exhibit 50% mortality to BPH. Nonetheless, low mortality (<60%) was observed, even at the highest concentration (10,000 ppm), stipulating that *A. conyzoides* only possesses a little toxicity towards the BPH. It was also noticed that at lower concentrations (1,000 ppm and below), the mortality was slow to develop, whereas the extract only showed its effect 72 hr after exposure time.

Similarly, a highly significant interaction between the treatment concentration and exposure time was observed in *C. odorata*. Nymph mortality increased substantially from 24 to 72 hr after exposure as treatment concentration increased (Figure 1). Less than 50% mortality occurred at 24 and 48 hr of



(a)



(b)

Figure 1. Interaction of concentration-time-mortality of the 3rd - 4th instar brown planthopper nymphs treated with (a) *Ageratum conyzoides* and (b) *Chromolaena odorata* methanol extract. The curves were fitted into a 4-parameteric sigmoidal hill curve using SigmaPlot 14 software. The standard error of the means was presented by vertical bars (n = 4)

exposure, even at the highest concentration (10,000 ppm). Mortality increased gradually at 72 hr of exposure time, where the highest nymph mortality (77.50%) was achieved at the highest concentration (10,000 ppm), indicating that *C. odorata* possesses a promising insecticidal compound toward the BPH.

Mallotus paniculatus methanol leaf extract was screened against BPH in eight concentrations, including the control. Highly significant mortality was observed between the treatment concentrations and between the exposure times (Table 3), while no significant interaction effect was observed. Low mortality was observed in the lowest four concentrations, from 0 (control) to 7,000 ppm, with less than 45% mortality. At the same time, the insecticidal effect was observed to be high (>45% mortality) in the four highest concentrations (8,000 to 12,000 ppm). Extension of the exposure time also increased the killing effect of the plant extract to approximately 1.8-fold from 24 to 72 hr.

The insecticidal activity of different plant extracts varies significantly, depending on the plant species and compatibility of the extraction solvent with the plant. Furthermore, insecticidal activity increased when treatment concentration increased simultaneously with the exposure times (Ahmed et al., 2020). Most synthetic insecticides can cause acute mortality. On the other hand, insects treated with plant extract had a slower mortality rate, whereas the extract did not cause death acutely. According to Ali et al. (2017), this phenomenon was due to some behavioral or physiological alterations, commonly known as sublethal or non-lethal effects experienced by the test insect. The dose/concentration the insect was exposed to would differ greatly over space and time. In a study conducted by Senthil-Nathan

Table 3
Mean mortality of 3rd and 4th instar brown planthopper (BPH) nymphs treated with Mallotus paniculatus *methanol extract*

	Percentage (%) mortality of BPH			
Treatment (ppm)	**24 hr**	**48 hr**	**72 hr**	***Mean regardless of time ± SE**
Control (Water)	2.50a	7.50a	7.50a	5.83 ± 1.93e
5,000	20.00b	30.00ab	47.50ab	32.50 ± 4.79d
6,000	25.00a	35.00a	50.00a	36.67 ± 5.27cd
7,000	32.50b	50.00a	50.00a	44.17 ± 3.13bcd
8,000	32.50c	47.50b	62.50a	47.50 ± 5.11abc
9,000	35.00b	47.50ab	65.00a	49.17 ± 4.84abc
10,000	45.00b	57.50ab	70.00a	57.50 ± 4.29ab
12,000	47.50a	60.00a	72.50a	60.00 ± 5.08a
*Mean regardless of treatment ± SE	30.00 ± 2.94c	41.88 ± 3.34b	53.13 ± 4.05a	

Note. Means followed by the same alphabet within columns (hour) are not significantly different by Tukey's test at $p = 0.05$. Means followed by the same alphabet, regardless of time (within column) or treatment (within row), are not significantly different by Tukey's test at $p = 0.05$

et al. (2009), the effective concentration of two botanical insecticides, Parker Oil™ (Parker Hannifin Corporation, USA) and Neema® (Neema International, USA) took more than 48 hr to kill 80% of the BPH. In the current study, the inhibitory effect of the plant extracts on BPH was observed every 24 hr for 3 consecutive days. After 72 hr, the rice plants in the control treatment (distilled water) also began to die due to rapid consumption by the BPH, resulting in a decrease in the BPH due to the depletion of food sources. It is also equally notable that all the formulated commercial insecticides have a specific active ingredient/premix of active ingredients, which have been proven through intensive evaluation to possess effective toxicity towards the target pest insect at the registered recommended rate. Hence, an investigation of the chemical compounds in the selected weed species was also done as an antecedent step in isolating the potential primary active ingredient candidate for bio-insecticide.

LC_{50} of 3rd and 4th Instar Nymphs of BPH in Methanol Extracts

LC_{50} is a concentration of a substance in a controlled environment that is expected to kill 50% of tested organisms in a given population (Duffus, 1993). An LC_{50} assessment was done on the mortality at 72 hr after exposure to the plant extracts to evaluate the effective toxicity effect of the methanol extracts. The results demonstrated that *C. odorata* significantly recorded the lowest LC_{50} value as compared to *M. paniculatus* and *A. conyzoides* (Table 4).

In a study by Lawal et al. (2014), the methanol extract of *C. odorata* becomes highly toxic even at very low concentrations, resulting in an LC_{50} value of 0.0039% (39 ppm) to kill *Sitophilus zeamais*. In comparison with another study by Matur and Davou (2007), *C. odorata* plant extract was able to cause low LC_{50} value against *Simulium* larvae, which was 0.001 mg/ml (100 ppm) concentration. On the contrary, methanol extract of *Ocimum gratissimum* only caused 73.92% mortality at 10,000 ppm (M. S. Kumar et al., 2017), similar to what was observed in this study for *C. odorata*. In plant extraction by solvent, the ability of certain plants to exhibit high mortality at a lower rate was due to several factors, such as pre-extraction preparation (plant part and sample preparation) and solvent choices (Azwanida, 2015). The solvent selection was of great significance (Khan et al., 2017), as it would ultimately dictate the extraction compounds that play a key role in affecting insect bioassays. This selection

Table 4
Median lethal concentration (LC_{50}) value after 72 hr exposure

Sample	Slope ± SE	LC_{50} (ppm)	95% confidence limits (ppm)	Chi-square (X^2)
Ageratum conyzoides	0.86 ± 0.28	21,940a	9,469 ~ 325,720	0.99
Chromolaena odorata	0.63 ± 0.14	977b	368 ~ 2,151	1.46
Mallotus paniculatus	2.12 ± 0.69	6,549a	4,074 ~ 8,039	0.90

Note. LC_{50} value with the same alphabet are not significantly different by Tukey's test at $p = 0.05$

not only determines the type and quality of extracted bioactive compounds but also directly influences insect mortality during the bioassay process (Fotsing et al., 2021).

Emulsion Formulation

The pseudoternary phase diagram was constructed to compare the surfactant, oil, and water ratio to obtain a single-phase (isotropic) region while conducting the emulsion formulation. Three pseudoternary phase diagrams were attained, comprising three different oils as carriers with similar surfactant types (Table 5). The three-component system is shown in Figure 2 (Emersense® AM 8025/PKOE/water), Figure 3 (Emersense® AM 8025/Edenor®/water) and Figure 4 (Emersense® AM 8025/Edenol® SP100/water). A continuous single-phase diagram of the isotopic region was observed in the green area, which is <20% of the total area for the three different formulations, while the blue/pink/orange region refers to milky and multi-layer (multi-phase). Emersense® AM 8025/PKOE/water system and Emersense® AM 8025/Edenor®/water system exhibited similar percentages of isotropic region (Figures 2 and 3). Nevertheless, Emersense® AM 8025/Edenol® SP100/water system resulted in a very trifling isotropic region (Figure 4). The solubilization capacity and areas of the emulsion systems were due to the structural similarity between the lipophilic

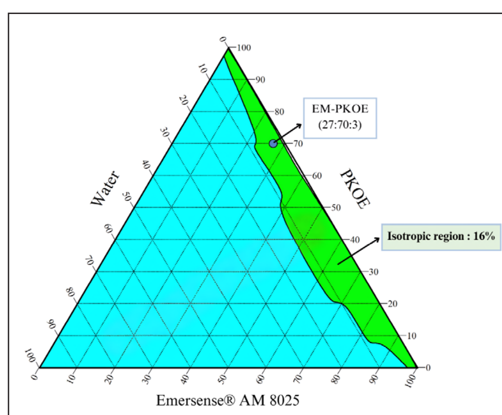


Figure 2. Phase diagram of Emersense® AM 8025: PKOE: Water system
Note. EM-PKOE = Emersense® AM 8025/palm kernel oil ester/water

Table 5
Percentage (w/w) compositions of surfactants, oil, and water contained in the emulsion formulations system without plant extract

Formulation (w/w)	Emersense® AM 8025[a] (%)	PKOE[b] (%)	Edenor®[c] (%)	Edenol® SP100[d] (%)	Water[e] (%)
EM-PKOE	27	70	-	-	3
EM-ED_1	30	-	20	-	50
EM-ED_2	25	-	15	-	60
EM-ED_3	20	-	10	-	70
EM-ED_4	15	-	5	-	75
EM-$EDSP_1$	29	-	-	11	60
EM-$EDSP_2$	25	-	-	5	70

Note. [a] = Non-ionic surfactant; [b,c,d] = Oil carriers; [e] = Solvent; EM-PKOE = Emersense® AM 8025/palm kernel oil ester/water; EM-ED = Emersense® AM 8025/Edenor®/water; EM-EDSP = Emersense® AM 8025/Edenol® SP100/water

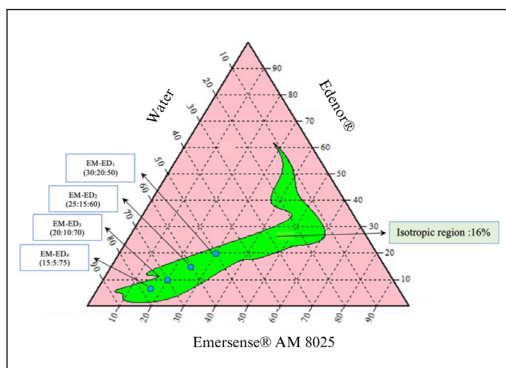


Figure 3. Phase diagram of Emersense® AM 8025: Edenor®: Water system
Note. EM-ED = Emersense® AM 8025/Edenor®/water

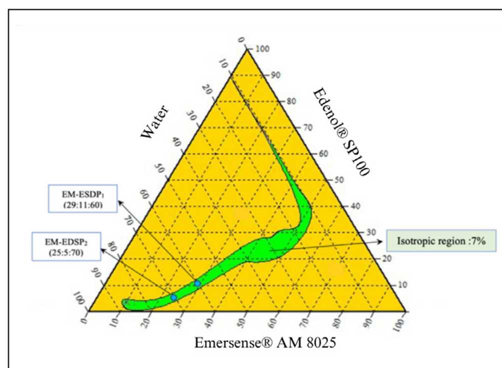


Figure 4. Phase diagram of Emersense® AM 8025: Edenol® SP100: Water system
Note. EM-EDSP = Emersense® AM 8025/Edenol® SP100/water

tail of surfactant and the oleyl group of the PKOEs (Mahdi et al., 2011).

There are three types of surfactants: ionic, anionic, and non-ionic. The most commonly used surfactant in pesticide formulation is non-ionic (Appah et al., 2020). A non-ionic surfactant was chosen in this study because of the less toxicity and irritation it imposes upon plants, animals, and humans, as well as the impact on the environment (Azeem et al., 2009; Pulce & Descotes, 1996). The required amount of surfactant also plays an important role in conducting emulsion formulation as a high concentration of surfactant could lead to phytotoxicity and also increase the production cost because surfactants are the most expensive element in an emulsion formulation compared to oil (Pratap & Bhowmick, 2008). Surfactants below 30% were suitable for emulsion formulation, especially in formulating microemulsions (Pratap & Bhowmick, 2008) and nanoemulsions (Choupanian et al., 2017).

Several points were selected from the isotropic region (green-colored region) according to the criteria of an adequate amount of surfactant (<30%). One point from Emersense® AM 8025/palm kernel oil ester/water coded as EM-PKOE, four points from Emersense® AM 8025/Edenor®/water coded as EM-ED, and two points from Emersense® AM 8025/Edenol® SP100/water coded as EM-EDSP were selected within the isotropic region. Based on the chosen points, only EM-PKOE emulsion had the least water content (3%) and the highest oil content (70%) in the formulation. In contrast, emulsions containing either Edenor® or Edenol® SP100 were observed to have higher water content (>50%) than oil content (<20%).

According to this finding, the methanol crude extract of *C. odorata* showed low miscibility when mixed with water alone. Thus, the incorporation of 5% *C. odorata* methanol crude extract as the active ingredient into the seven emulsion systems was investigated. Among the seven points from the three different emulsion systems,

only EM-PKOE emulsion appeared compatible with the 5% *C. odorata* methanol crude extract as it achieved a stable emulsion with no phase separation. In contrast, EM-ED and EM-EDSP showed incompatibility with 5% *C. odorata* methanol crude extract as they failed to remain single-phased. The compatibility of the extract when incorporated with the emulsion might be due to the compounds present in the extract. It is well known that oils are widely used as emulsion-type pesticide adjuvants to enhance the spread of droplets on plant surfaces with the possibility to penetrate lipophilic regions in leaf cuticles, thus increasing absorption of leaf extract into plant cells (Wang et al., 2018).

Characterization of the Macroemulsion Formulation

Stability of Formulation

EM-PKOE emulsion, which contained 5% *C. odorata* extract, was tested for its stability at room temperature (25 ± 2°C) for 30 days and thermostability at 54°C for 14 days. Based on visual observation, there was no phase separation, creaming, or sedimentation in the emulsion at room temperature and after the heat test (54°C). Hence, this emulsion exhibited good stability, which met the requirements for insecticide formulation according to the Food and Agriculture Organization of the United Nations and the World Health Organization (2010).

Zeta Potential

The zeta potential determined for EM-PKOE using LDE were found to be negatively charged (-62.70 mV). Zeta potential is often used as an indicator of droplet stability, where values more positive than +30 mV and more negative than -30 mV (Han et al., 2008) exhibit strong repelling force, indicating good stability against coalescence (Kadu et al., 2011). A net charge of either sign on the particle surface repels the particles electrostatically, preventing aggregation, coagulation, or flocculation to a certain extent (Sis & Birinci, 2009). Therefore, the zeta potential determined in this study demonstrates emulsion stability, as it exceeds -30 mV. The zeta potential is the difference in potential between the mobile dispersion medium and the stationary layer or fluid in which the dispersed particle is suspended (Lu & Gao, 2010). In other words, it is the assessment or determination of a colloidal system's surface charge (Shnoudeh et al., 2019).

Particle Size and PDI

The particle size distribution of diameters of the dispersed phase of EM-PKOE was determined using the Malvern instrument and the DLS technique. Based on the analysis, the mean particle size of the EM-PKOE emulsion was 444.40 nm in diameter, considered a conventional emulsion or macroemulsion. Macroemulsions have particle sizes >200 nm in diameter, and they are optically turbid as in EM-PKOE because the droplet size is comparable to the wavelength of light, hence scattering the incident light and making it appear opaque. In addition, macroemulsion is kinetically stable and thermodynamically metastable. The advantage of this emulsion

type is also comparable to nanoemulsion, where it is very stable to temperature and pH changes (Aswathanarayan & Vittal, 2019).

The determination of particle size in an emulsion is one of the very important physical characteristics because it can distinguish the emulsion types and the stability of the emulsion (Mudalige et al., 2019). A large particle size in an emulsion was most likely due to a higher oil content, resulting in a larger droplet size in the emulsions (Zheng et al., 2020). The higher oil content, therefore, describes the emulsion type as a "water-in-oil" (w/o) emulsion where water is the dispersed phase that is distributed into the continuous phase (oil). Moreover, the droplet size distribution will also contribute to the determination of the PDI of an emulsion.

The PDI is basically a depiction of the size distribution heterogeneousness by the instruments of DLS within a sample. This index is dimensionless with value ranges from 0.00 to 1.00 for a sample that is totally uniform/homogeneous and for a sample that is not perfectly uniform/not homogeneous in particle size distributions accordingly (Danaei et al., 2018). In this study, the PDI analyzed for EM-PKOE was 0.60. According to Nobbmann (2017), a PDI value >0.40 is categorized as a broad polydisperse distribution, meaning the sample particles varied in size. Polydispersion may arise because of the distribution or agglomeration of a sample during isolation or analysis (Mudalige et al., 2019) and might be due to the solvent used during the dilution of the sample, as mentioned by Motwani et al. (2006).

The higher the PDI, the larger the particle size. The particle size distribution is reflected in the PDI value as well. Samples with a larger range of particle sizes have higher PDI values, whereas samples with equally sized particles have lower PDI values (Masarudin et al., 2015). There is no PDI limit because the purpose of the formulation determines it. For example, it is feasible to have a low PDI value since it reflects particle dispersity in a homogeneous sample, which means that the particles are smaller and more uniform, and therefore, if penetration is necessary to infiltrate a cell, it is more efficient (Danaei et al., 2018).

Viscosity

The viscosity test that was carried out on EM-PKOE emulsion containing 5% *C. odorata* extract showed a range of 33.09 to 35.29 mPa.s, which is considered very viscous, while the viscosity of water is only about 0.90 mPa.s at room temperature (Foliadi et al., 2018). Viscosity depends on surfactant type, surfactant-to-oil (S/O) ratios, and oil concentration. Increased S/O and oil concentration will result in high viscosity (Chanana & Sheth, 1995). Similarly, reducing the amounts of surfactant or decreasing water volume can also increase interfacial tension between water and oil, producing a more viscous emulsion (Marzuki et al., 2019).

Mortality Bioassay of Emulsion Formulation Containing Crude Extract

Toxicity effect determination of EM-PKOE macroemulsion containing 5% *C. odorata*

leaves extract was performed against the 3rd and 4th instar nymphs of BPH. Based on the analysis, BPH cumulative mortality was significantly influenced by the treatment concentration and the days of exposure. Further analysis was carried out to observe the interaction between the two factors (treatment concentrations and days of exposure), and a highly significant interaction was recorded between the treatment concentrations and days of exposure. Nymph mortality increased as treatment concentrations and days of exposure were increased. Higher concentration and more time were needed to achieve higher nymph mortality.

Based on these findings, the formulated EM-PKOE macroemulsion with *C. odorata* leaf extract had a toxic effect against BPH 3rd and 4th instar nymphs. Nymph mortality escalated as exposure time increased regardless of the treatment concentrations (Table 6). Furthermore, nymphs' mortality was also influenced by the macroemulsion concentration, where higher concentration resulted in a higher mortality percentage regardless of the exposure time. Hence, it can be concluded that treatment concentration and exposure time apparently showed significant interaction, where an increase in exposure time and treatment concentration would cause an increase in mortality percentage (Bouda et al., 2001).

The EM-PKOE emulsion with *C. odorata* methanol extract displayed higher efficacy against the BPH nymphs, especially at higher concentrations (5,000 and 10,000 ppm) as compared to the positive controls, namely Regent® (fipronil) and Neemix® (azadirachtin), the commercial chemical and botanical insecticide, respectively. Overall, during the final observation (48 hr), there was no significant mortality among the four higher concentrations (500, 1,000, 5,000, and 10,000 ppm) and the positive controls, where the EM-PKOE emulsion was able to kill >80% of the nymphs at lower concentration of 500 ppm. Interestingly, at 1,000 ppm, mortality of the emulsion was comparable to that of the positive controls, which was also evident in every hour of observation. Fipronil used in this study is a broad-spectrum insecticide, which is highly effective against sucking and chewing insects. It has been widely used for the control of many species of soil and foliar insects of various agricultural crops, but indiscriminate use of this insecticide in recent times has resulted in the development of resistance in planthoppers (N. Kumar et al., 2019). On the other hand, azadirachtin is a potent antifeedant (feeding deterrent) botanical insecticide that often results in starvation, ultimately killing the insect (Heong et al., 2013).

The emulsion formulation greatly affected and influenced the nymphs' mortality in this study. The incorporation of *C. odorata* into the EM-PKOE emulsion substantially improved the extract's efficacy. As an example, at the highest concentration of 10,000 ppm, a total control (100%) was improved by 22.50% at a shorter exposure time (48 hr) in the formulated emulsion as compared to the *C. odorata* crude extract alone (77.50% mortality after 72 hr of exposure) against the BPH nymphs.

Table 6
Interaction of treatment concentration-time-mortality of 3rd and 4th instar nymphs of Nilaparvata lugens treated with EM-PKOE macroemulsion containing 5% Chromolaena odorata extract

	Percentage (%) mortality of BPH								
Treatment	**6 hr**	**12 hr**	**18 hr**	**24 hr**	**30 hr**	**36 hr**	**42 hr**	**48 hr**	***Mean regardless of time ± SE**
Control (Fipronil)	52.50b	60.00bc	75.00b	82.50ab	90.00a	95.00a	97.50a	97.50a	81.30 ± 3.20bc
Control (Azadirachtin)	45.00b	70.00b	77.50b	95.00a	95.00a	97.50a	97.50a	97.50a	84.40 ± 3.42b
Control (Water)	0.00c	0.00d	0.00e	0.00c	0.00d	5.00d	10.00c	12.50c	3.40 ± 1.15f
100 ppm	7.50c	15.00d	15.00d	17.50c	22.50c	25.00c	25.00c	30.00b	19.70 ± 2.03e
500 ppm	37.50b	47.50c	55.00c	60.00b	62.50b	72.50b	77.50b	85.00a	62.20 ± 3.32d
1,000 ppm	55.00b	65.00bc	72.50b	85.00ab	85.00a	85.00ab	92.50ab	92.50a	79.00 ± 2.43c
5,000 ppm	77.50a	90.00a	97.5.0a	100.00a	100.00a	100.00a	100.00a	100.00a	95.60 ± 1.62a
10,000 ppm	85.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	100.00a	98.10 ± 0.95a
*Mean regardless of treatment ± SE	45.00 ± 5.18e	55.90 ± 5.90d	61.60 ± 6.21d	67.50 ± 6.72c	69.40 ± 6.58bc	72.50 ± 6.32abc	75.00 ± 6.20ab	76.90 ± 5.98a	

Note. EM-PKOE = Emersense® AM 8025/palm kernel oil ester/water; BPH = Brown planthopper; Means followed by the same alphabet within columns (hour) are not significantly different by Tukey's test at $p = 0.05$. Means regardless of time (within column) and means regardless of treatment (within row) followed by the same alphabet are not significantly different by Tukey's test at $p = 0.05$

LC_{50} of 3rd and 4th Instar Nymphs of BPH in Emulsion Formulation

The LC_{50} values were analyzed to determine the effective concentrations for the emulsion that caused 50% mortality of the nymphs' population at different exposure times, as shown in Table 7. Regardless of concentration, increased toxicity (lower LC_{50} value) was recorded as the exposure time extended from 6 to 48 hr. At the earliest exposure time (6 hr), the LC_{50} was estimated to occur at 1,061 ppm, similar to the result obtained in the non-formulated bioassay where at 1,000 ppm, 55% mortality was achieved. The LC_{50} was reduced by half from the beginning of the observation until the final day of observation. Even though 48 hr showed the lowest LC_{50} value (220 ppm), it was not significantly different with 24 hr (320 ppm), 30 hr (280 ppm), 36 hr (270 ppm), and 42 hr (260 ppm). As shown in Table 3, the LC_{50} value for non-formulated *C. odorata* was 977 ppm on the final day of observation (72 hr of exposure), which was higher than the formulated *C. odorata* in EM-PKOE emulsion on the final day of observation (220 ppm after 48 hr of exposure). Evidently, incorporating 5% *C. odorata* into the EM-PKOE emulsion increased the extract's effectiveness in controlling BPH nymphs by 63.20%, similar to what was observed by Ezena et al. (2016), where the efficacy of *C. odorata* extract with the addition of sunflower oil, soap, roasted cocoa pods, red palm oil, coconut oil, sea salt, and shea butter in the formulation substantially increased in controlling diamondback moth (*Plutella xylostella* L.).

Chemical Analysis of *C. odorata*

The GC-MS analysis of *C. odorata* leaf methanol extract recorded 65 compounds, and the identified components are presented in Table 8. The most abundant component in *C. odorata* was sesquiterpenes (24.14%), followed by fatty acid (14.65%), triterpenes (7.94%), flavonoids (6.05%), sterol (5.19%),

Table 7
Variation of LC_{50} (ppm) with respect to the duration of exposure of 3rd and 4th instar Nilaparvata lugens *nymphs to the EM-PKOE emulsion formulated with* Chromolaena odorata *leaves extract*

Exposure time (hr)	Slope ± SE	LC_{50} (ppm)	95% confidence limits (ppm)	Chi-square (X^2)
6	1.18 ± 0.16	1,061a	710 ~ 1,560	1.56
12	1.51 ± 0.19	530b	370 ~ 740	1.73
18	1.78 ± 0.23	420bc	300 ~ 560	0.51
24	2.04 ± 0.28	320bcd	230 ~ 430	1.03
30	1.88 ± 0.26	280cd	200 ~ 380	1.17
36	1.95 ± 0.31	270cd	170 ~ 380	0.54
42	2.38 ± 0.43	260cd	160 ~ 360	0.05
48	2.31 ± 0.42	220d	130 ~ 310	0.56

Note. LC_{50} is the concentration required to result in a 50% effect. Values with the same alphabet within a column are not significantly different by Tukey's test at $p = 0.05$; EM-PKOE = Emersense® AM 8025/palm kernel oil ester/water

Table 8
Chemical constituents identified in the leaves of Chromolaena odorata *from methanol extract*

Compound class**	Retention time*	Relative percentage	Compound name
Sesquiterpenes	26.86	0.16	α -Cubebene
	28.07	1.04	α -Cubebene
	30.03	1.97	(E)-Caryophyllene
	30.43	0.78	Germacrene-D
	31.51	0.55	α -Humulene
	32.49	0.69	γ -Cadinene
	32.69	0.26	Germacrene-D
	33.50	0.23	α -Muurolene
	34.48	2.04	δ -Cadinene
	36.69	1.02	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane
	37.01	1.05	Caryophyllene oxide
	39.81	0.39	Cadin-4-en-10-ol
	46.54	2.81	Neophytadiene
	47.41	0.62	Neophytadiene
	48.03	0.93	Neophytadiene
	64.04	0.90	(+)-Sativen
	63.13	4.48	Aromadendran ('1')
	64.68	3.42	(+)-aromadendrene
	65.56	0.30	1-alpha-, 10-alpha-epoxy-Amorph-4-ene
	66.14	0.50	Palustrol
Fatty acids	38.04	0.34	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]
	49.57	0.18	Hexadecanoic acid, methyl ester
	50.90	4.38	n-Hexadecanoic acid
	55.12	0.18	Linoleic acid, methyl ester
	55.32	0.24	Methyl linolenate
	56.42	2.68	9,12-Octadecadienoic acid (Z,Z)
	56.66	4.76	9,12,15-Octadecadienoic acid (Z,Z)
	57.26	0.46	Octadecanoic acid
	72.25	0.53	1-Heptacosanol
	77.17	0.90	Lignoceric alcohol
Triterpenes	75.38	3.63	Squalene
	89.51	3.30	Olean-12-en-3-one
	90.95	0.33	β-Amyrin
	92.42	0.68	Lupeol acetate
Flavanoids	72.10	0.90	5-Hydroxy-4',7-dimethoxyflavanone
	83.54	2.84	Flavone, 4',5,6,7-tetramethoxy
	84.41	2.31	Flavone, 3,4',5-trihydroxy-3',7-dimethoxy

Table 8 *(continue)*

Compound class**	Retention time*	Relative percentage	Compound name
Sterol	85.68	2.51	Stigmasterol
	87.13	2.68	β-Sitosterol
Diterpenes	34.09	0.32	γ -Amorphene
	55.85	1.70	Phytol
Vitamin E	82.50	1.15	dl-alpha-Tocopherol
Pyrrole	3.48	0.19	Methyl pyrrole
Unknown	60.92	0.30	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester
	66.00	0.31	Carbonic acid, 2-dimethylaminoethyl ethyl ester
	75.71	0.83	Ethyl 4-hydroxy-3-methoxyphenyl acetate

Note.* = Retention time on the Rxi-5MS silica capillary column; ** = Search using Chemical Entities of Biological Interest (ChEBI) web-based application (Hastings et al., 2016)

diterpenes (2.02%), vitamin E (1.15%), and pyrrole (0.19%). Aromadendran ('1') (4.48%) was the main constituent of sesquiterpenes, followed by neophytadiene (4.36%), (+)-aromadendrane (3.42%), δ-cadinene (2.04%), (E)-caryophyllene (1.97%), α-cubebene (1.20%), caryophyllene oxide (1.05%), and germacrene-D (1.04%). As for fatty acid, the main constituent was 9,12,15-octadecadienoic acid (Z,Z) (4.76%), followed by *n*-hexadecanoic acid (4.38%), and 9,12-octadecadienoic acid (Z,Z) (2.68%). In triterpenes, the main components were squalene (3.63%) and olean-12-en-3-one (3.30%). Meanwhile, only stigmasterol (2.51%) was found to be sterol, while in flavonoid, the main components detected were flavone, 4',5,6,7-tetramethoxy (2.84%) and flavone, 3,4',5-trihydroxy-3',7-dimethoxy (2.31%). At the same time, phytol (1.70%) was the most abundant component found as a diterpenoid. Previously, Akinmoladun et al. (2007) found that the leaf extract of *C. odorata* contained terpenes, flavonoids and steroids. Jasnie (2009) and Joshi (2013) also observed the presence of germacrene-D, caryophyllene oxide, hexadecenoic acid, stigmasterol, γ -cadinene, δ -cadinene, and hexadecenoic acid in *C. odorata* plant extract.

Several studies on *C. odorata* extracts, which pose insecticidal properties, were also reported by other researchers against other insects. *Chromolaena odorata* extract effectively controlled the larval and pupal stages of the malaria vector (*Anopheles gambiae*) (Ileke & Olabimi, 2019). The presence of stigmasterol in *C. odorata* was identified to be responsible for the larvicidal activity in *Culex quinquefasciatus* and *Aedes aegypti*, in which the compounds that inhibit the acetylcholinesterase activity in the test insects possessed neurotoxicity action (Gade et al., 2017). Similar to this study, stigmasterol was also found in *C. odorata* methanol extract, possibly influencing BPH's mortality.

In addition, Langenheim (1994) also stated that plant terpenes were often reported as having anti-herbivore defenses. As an example, germacrene-D was previously studied to have deterrent effects against herbivores (Kiran & Devi, 2007), repellent activity against ticks (Birkett et al., 2008) and aphids (Bruce et al., 2005), as well as having insecticidal activity against mosquitoes (Langenheim, 1994). Another biological activity was also found in phytol (diterpene), which successfully controlled severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* (Saha & Bandyopadhyay, 2020), whereas squalene (triterpene) was responsible as an antioxidant (Huang et al., 2009) and antitumor (Huang et al., 2009; Senthilkumar et al., 2006).

CONCLUSION

The solvent type significantly influenced the extraction yield, with methanol surpassing hexane by 69%. *Chromolaena odorata* methanol extract showed a greater mortality rate and a lower LC_{50} value after 72 hr against the 3rd and 4th instar nymphs of BPH during the screening bioassay. An improved efficacy by 56% mortality was observed in the formulated bioassay when *C. odorata* methanol extract was successfully incorporated as the a.i. into EM-PKOE macroemulsion. Furthermore, insect mortality could also be attributed to the bioactive content of the leaf extract. *C. odorata* methanol extract was well known for its insecticidal effects against a variety of agricultural pests and diseases, especially with the presence of the terpenes group. Although macroemulsion showed a significant reduction in mortality and an increase in efficacy, formulation improvements are necessary as the world is increasingly moving towards nanoemulsion. Hence, exploration of the isolation and formulation of specific chemical compounds will enable the development of a new formulation of bio-insecticide for crop protection purposes.

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