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Physiological Interference of Nanoemulsion-Formulated Botanical Insecticide Para-Menthane-3,8-Diol on *Spodoptera frugiperda*

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Abstract

Para-menthane-3,8-diol (PMD), derived from citronellal of citronella plants, has potential as an eco-friendly botanical insecticide for controlling *Spodoptera frugiperda*. This study aimed to evaluate the physiological interference caused by a PMD nanoemulsion formulation on *S. frugiperda*. Bioassays were conducted using the leaf-dip method. Data were analyzed using analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) test with Statistix 8 and Duncan's multiple range test at a 5% significance level using SPSS version 25. Probit analysis with POLO PLUS was performed to determine LC_{25} and LC_{50} values. The results showed that PMD nanoemulsion induced significant physiological interference in *S. frugiperda*, as indicated by increased mortality with LC_{25} and LC_{50} values of 0.15% and 0.48%, respectively. At a concentration of 0.75%, the treatment disrupted development by reducing pupation and adult emergence to 30% and altering the sex ratio to 1 male: 0.22 female. Sublethal interference was evident through strong antifeedant activity (62.88%) and pronounced changes in physiological parameters, including consumption rate (0.0328 mg/day), growth rate (0.0023 mg/day), and efficiency of ingested food (6.0029 mg/day). Overall, the PMD nanoemulsion formulation interfered with survival, feeding behavior, growth, and developmental physiology of *S. frugiperda*, highlighting its potential as an eco-friendly botanical insecticide.

Keywords: Antifeedant, assimilation, disruption, efficiency, eco-friendly

Introduction

Maize (*Zea mays*) is an important food crop used as a staple carbohydrate source after rice and wheat and widely utilized as animal feed and industrial raw material. In 2022, national maize production reached 23.1 million tons, while domestic demand during 2023–2024 was estimated at 35.3 million tons (BPS, 2022). One of the main constraints to maize production is damage caused by insect pests, particularly the fall armyworm, *Spodoptera frugiperda* (Bagariang et al., 2020; Yurina et al., 2023; Syafria et al., 2023). This pest has been reported across several regions of Indonesia (BBPOPT, 2019) and primarily attacks the growing point, causing severe damage and potential plant growth failure (CABI, 2019). Infestation levels during the vegetative stage may reach 6–90% (Nelly et

al., 2022), leading farmers to rely heavily on synthetic insecticides. However, prolonged insecticide use can disrupt ecosystem stability and cause environmental degradation, highlighting the need for sustainable alternatives.

Citronella (*Cymbopogon nardus*) is a promising plant source for botanical insecticide development due to its high content of secondary metabolites, particularly citronellal (27.87%), geraniol (22.77%), and citronellol (11.85%) (Toledo et al., 2016). Among these compounds, citronellal is the dominant component and has been reported to inhibit acetylcholinesterase (AChE), with an IC_{50} value of 18.4 mg/mL, as well as ATPase, with an IC_{50} value of 13.26 mg/mL, in *Sitophilus oryzae* (Saad et al., 2017). The mode of action of citronellal on AChE involves prolongation of the depolarization phase by maintaining sodium channels in an open state for a longer duration, resulting in continuous nerve impulse transmission and impaired neural regulation (Nugroho, 2011). Additionally, citronellal inhibits ATPase activity, resulting in reduced cellular energy production and disrupted ion transport processes (Abou-Taleb et al., 2015). Citronellal has also demonstrated insecticidal activity against *Tribolium castaneum*, with an LC_{50} value of 1.2% (Bossou, 2015). However, its overall insecticidal efficacy remains relatively limited, indicating the need for further fractionation or formulation strategies to enhance the bioactivity of citronellal-based insecticides.

Para-menthane-3,8-diol (PMD) is a synthetic derivative of citronellal (Sulaswatty et al., 2019) and is widely recognized for its repellent properties (Syafi'i et al., 2020). PMD has been reported to inhibit feeding activity by 49.1% in *Aedes aegypti* (Lee et al., 2018) and to induce larval mortality with an LD_{95} value of 0.25% (Goodyer et al., 2020). However, to enhance its insecticidal performance and broaden its agricultural application, PMD activity requires optimization through appropriate formulation strategies (Le et al., 2023). Standard formulations used in agriculture include emulsifiable concentrates (ECs) and wettable powders (WPs). EC formulations offer ease of application and do not leave visible residues on plant surfaces; however, they may pose risks of phytotoxicity. In contrast, WP formulations are generally safer for plants and easier to store; however, they require continuous agitation during application and often leave noticeable residues on treated plants (Hazra et al., 2019).

Nanoemulsion technology has emerged as an innovative approach for botanical insecticide formulation, as it produces nanosized particles that enhance penetration into leaf tissues, maintain good storage stability, and reduce sedimentation. Nano-emulsions typically consist of aqueous and organic phases at a 9:1 ratio with an active ingredient concentration of approximately 5% (Lina et al., 2021), and their use can reduce the required amount of active ingredient by up to 12.4-fold compared with crude extracts (Lina et al., 2025). Nanoemulsion formulations combining citronella hydrosol and *Piper aduncum* extract have been reported to effectively control *Spodoptera frugiperda* larvae, with an LC_{95} value of 1.29 (Mardha, 2023), and *Crocidolomia pavonana* larvae, with an LC_{95} value of 0.97 (Rani, 2023).

The response of *S. frugiperda* to insecticides is strongly influenced by biochemical adaptations in the digestive system and food assimilation processes. Therefore, assimilation studies are essential for understanding how insecticidal com-pounds affect insect growth, survival, and population dynamics. Host plant suitability directly determines insect fitness and

reproductive performance. High-quality and sufficient host plants allow insects to attain optimal physiological conditions, whereas unsuitable or toxic hosts reduce food consumption, induce suboptimal feeding behavior, or lead to starvation, ultimately resulting in growth inhibition, developmental disorders, or mortality. Lina (2014) reported that the LC_{25} and LC_{50} concentrations of a *Tephrosia vogelii*: *Piper aduncum* extract mixture (1:5) disrupted relative growth rates and increased digestibility by 11.11%, which was interpreted as an adaptive physiological response to toxic compounds. Similarly, Tama (2019) demonstrated that nanoemulsions containing *T. vogelii* extract significantly reduced growth rates in *Crocidolomia pavonana* larvae, indicating metabolic disruption associated with the ingestion of toxicants. However, information regarding the physiological interference of PMD-based botanical insecticide nanoemulsions in *Spodoptera frugiperda* remains limited.

Based on these findings, this study aimed to determine the physiological effects of a PMD-based botanical insecticide nanoemulsion on *S. frugiperda*. The results are expected to provide a scientific basis for developing sustainable pest management strategies, particularly in maize cultivation.

Methods

The evaluation of the activity of botanical insecticide, nanoemulsion formulations containing parametandiol (PMD) was conducted at the Insect Bioecology Laboratory, Plant Protection Department, Faculty of Agriculture, Universitas Andalas, Padang, Indonesia, from October to December 2024.

Research Design

The activity of the PMD based botanical insecticide nanoemulsion against *S. frugiperda* was assessed through two experimental stages, namely toxicity assays and food assimilation assays. The study was arranged using an experimental method based on a Completely Randomized Design (CRD). The toxicity assay consisted of two stages: a preliminary test and a definitive test.

The preliminary test was carried out to determine the appropriate range of concentrations for the definitive toxicity assay and to establish the treatment concentrations used in the food assimilation assay. It used nanoemulsion concentrations of 0.00%, 0.50%, and 1.00%, each with 5 replications. Each replicate consisted of a petri dish containing 10 second-instar *S. frugiperda* larvae fed on 4×4 cm corn leaves dipped in the treatment solution for 3 seconds, dried, and placed in the dish. Treated leaves were provided for 2×24 h, and mortality was recorded daily until larvae reached the sixth instar. Surviving third-instar larvae were transferred to individual plastic cups.

The definitive toxicity assay was subsequently conducted to determine lethal concentration (LC) values. The food assimilation assay was performed using the residue-on-diet method on third-instar larvae of *S. frugiperda*, with treatment concentrations based on the LC_{25} and LC_{50} values obtained from the toxicity assay. The expected outcomes of the second stage of this study were to obtain information on the effects of the PMD-based botanical insecticide nanoemulsion on biological parameters of *S. frugiperda*, including larval mortality, pupal formation, adult emergence, sex ratio, and food assimilation capacity.

Preparation of Insect Diet

The diet used in this study consisted of sweet corn plants (*Zea mays* saccharata) at four weeks after planting. The corn plants were grown in 10-kg polybags containing a

growth medium composed of soil and fertilizer at a ratio of 4:1. Planting was carried out by making planting holes approximately 3 cm deep, into which three corn seeds were placed per polybag and then covered with soil. Corn plants were maintained through regular watering, application of NPK fertilizer, and mechanical control of plant pests to ensure plant quality as a food source for the test insects.

Rearing of Insects

Larvae of *Spodoptera frugiperda* were collected from corn fields in Kuranji, Padang City. Field-collected larvae were individually placed into plastic containers measuring 6 cm in diameter and 4 cm in height, each provided with fresh corn leaves as food. Each container housed only one larva to prevent cannibalism. The larvae were reared in the laboratory and fed fresh corn leaves until they reached the pupal stage.

The pupae were placed on sawdust as a substrate, collected, and transferred into mesh cages until adult emergence. Newly emerged adults were provided with cotton soaked in a 10% honey solution as a food source. Several pairs of male and female adults were placed in mesh cages and allowed to mate. Mated females were allowed to oviposit, and the hatched eggs constituted the first generation (F1) derived from field-collected parents. Rearing of *S. frugiperda* was continued until the third generation (F3), which was used as the test insect population in this study.

Nanoemulsion Formulation

The nanoemulsion formulation consists of aqueous phase and organic phase by spontaneous emulsification method. The aqueous phase by mixing distilled fair (55%) and surfactant tween 80 (15%) was homogenized using a magnetic stirrer for 30 minutes at 2500 rpm.

Preparation of the organic phase by mixing *para-Menthane-3,8-diol* (PMD) (15%), solvent (methanol) (14.5%), and defoamer (0.5%) by slowly dropping the organic phase into the aqueous phase using a mohr pipette while homogenizing using a magnetic stirrer for 45 minutes. The obtained formulation was transferred into a storage bottle and then covered with aluminum foil and stored in a refrigerator until used for testing.

Toxicity Test

Toxicity assays were conducted in two stages, namely a preliminary test and a follow-up test. The preliminary test employed nanoemulsion concentrations of 0.00%, 0.50%, and 1.00%, with five replicates per concentration. Each replicate consisted of a Petri dish containing ten second-instar Spodoptera frugiperda larvae fed with maize leaves (4 × 4 cm) that were immersed in the treatment solution for 3 s, air-dried, and provided for 2 × 24 h. Larval mortality was recorded daily until the larvae reached the sixth instar, and surviving third-instar larvae were transferred individually to plastic cups. Mortality data from the preliminary test were used to determine sensitive concentration ranges for the follow-up test.

The further test was conducted using the same procedure, with five replicates per concentration, to estimate LC_{50} and LC_{95} values. Mortality was recorded daily starting 24 h after treatment until the larvae reached the sixth instar; antifeedant activity was assessed on the first and second days after treatment, and pupal and adult emergence were observed daily from the pupal stage until adult emergence.

Food Assimilation

Testing food assimilation using the leaf residue method with LC_{25} and LC_{50} concentrations on *S. frugiperda* instar III larvae. Third instar *S. frugiperda* larvae were

weighed one by one as many as 10 larvae per repetition. The larvae were then placed into an insect rearing container. Feed was given according to the LC_{25} and LC_{50} concentration treatments. The leaves given as food were weighed one by one to determine their weight.

After treatment, the test larvae, remaining feed, and feces were weighed and then wrapped using aluminum foil one by one and dried in an oven at 100°C until constant weight. After constant weight, the larvae were weighed again to determine their dry weight. To estimate the initial dry weight, larvae and leaves were weighed to determine their wet weight. Larvae and leaves were wrapped in aluminum foil one by one and dried to constant weight. Larvae and leaves were weighed again to determine their dry weight.

Observation Variables

Larval mortality

Mortality of *S. frugiperda* larvae was observed after treatment feeding by dividing the number of dead test insects by the total number of test insects in each treatment. Larval mortality can be calculated using the following formula:

$$M = \frac{a}{b} x\ 100 \quad \text{..... (1)}$$

Description: M = Mortality (%), a = number of larvae dying, b = number of treated larvae

Antifeedant

The feeding inhibitory activity of mixed extracts was tested against *Spodoptera frugiperda* larvae by the residual method on leaves. Ten *S. frugiperda* instar II larvae per petri dish and allowed to eat the experimental leaves for 24 hours. The area of leaves eaten was immediately mapped on millimeter paper to calculate the amount of leaf area eaten. The effect of feeding inhibition without choice (PM) was calculated by the formula:

$$\text{Antifeedant} = \frac{a - b}{a} \text{x } 100 \quad \text{..... (2)}$$

Description: a = control leaf area eaten, b = treatment leaf area eaten

Pupae Formed

This observation is made by calculating the percentage of pupae that have formed. The percentage of pupae formed was calculated using the formula:

$$\text{Pupae formed } (\%) = \frac{p}{l} \text{x} 100 \quad \text{..... (3)}$$

Description: p = Number of larvae to pupae formed, l = Number of larvae applied

Adult Formed

This observation was made by calculating the mortality of adult from each treatment. The percentage of imago formed was calculated using the formula:

$$\text{Image formed} = \frac{m}{n} \text{x } 100 \quad \text{..... (4)}$$

Description: m = Number of larvae to imago formed, n = Number of larvae applied

Sex Ratio

The number of male and female imago was calculated from the number of adult that appear to determine the sex ratio between males and females.

Food Assimilation

The food assimilation data obtained consists of CR = consumption rate, AWL = average weight of larvae, RCR = relative consumption rate, GR = growth rate, RGR= relative growth rate, DC = digestibility, EFC = efficiency of food consumed, and EFD = efficiency of food digested (Lina et al., 2021).

$$\text{CR} = \text{initial feed} - \text{final feed} \quad \text{..... (5)}$$

$$\text{AWL} = \frac{\text{initial larvae+final larvae}}{1} \quad \text{..... (6)}$$

$$\text{RCR} = \frac{\text{Consumption rate}}{\text{initial larvae}} \quad \text{..... (7)}$$

$$\text{GR} = \frac{\text{final ralvae}-\text{initial larvae}}{\text{feeding period}} \quad \text{..... (8)}$$

$$\text{RGR} = \frac{\text{growth rate}}{\text{initial rate}} \quad \text{..... (9)}$$

$$\text{DC} = \frac{\text{consumption rate}-\text{feces}}{\text{consumption rate}} \text{ x } 100 \quad \text{.....(10)}$$

$$\text{EFC} = \frac{\text{final larvae}-\text{initial larvae}}{\text{feeding period}} \quad \text{..... (11)}$$

$$EFD = \frac{\text{late larvae} - \text{initial larvae}}{\text{consumption rate of adult}} \text{x } 100 \text{ ..... (12)}$$

Data Analysis

The data were analyzed by variance analysis (ANOVA) followed by the Least Significant Different (LSD) test using Statistics version 8 at the 5% level.

Results

Larval Mortality

Larval mortality of *S. frugiperda* observed in the toxicity assay indicated that increasing concentrations of the para-menthane-3,8-diol (PMD) nanoemulsion formulation were associated with a corresponding increase in larval mortality. All tested concentrations resulted in significantly higher mortality compared with the control treatment. The PMD nanoemulsion formulation caused larval mortality of *S. frugiperda* of 28% at a concentration of 0.25, 46% at 0.43, and 70% at 0.75 (Table 1).

Table 1. Mortality of *Spodoptera frugiperda* larvae after treatment in para-Menthane-3,8-diol nanoemulsion formulation

Concentration	Mortality (%) ± SD
0.75	70.00± 7.07 a
0.57	58.00± 8.36 ab
0.43	46.00±18.16 bcd
0.33	42.00± 4.47 cde
0.25	28.00± 8.36 ef
0.00	0.00± 0.00 g

Notes: Numbers followed by the same lowercase letter in the same column are not significantly different based on LSD test at the 5% level

The PMD nanoemulsion formulation exhibited an increasing pattern of larval mortality of *S. frugiperda* with increasing concentrations, and mortality increased from the first to the second day of observation. From the second day until the final observation, no further increase in larval mortality was recorded (Figure 1).

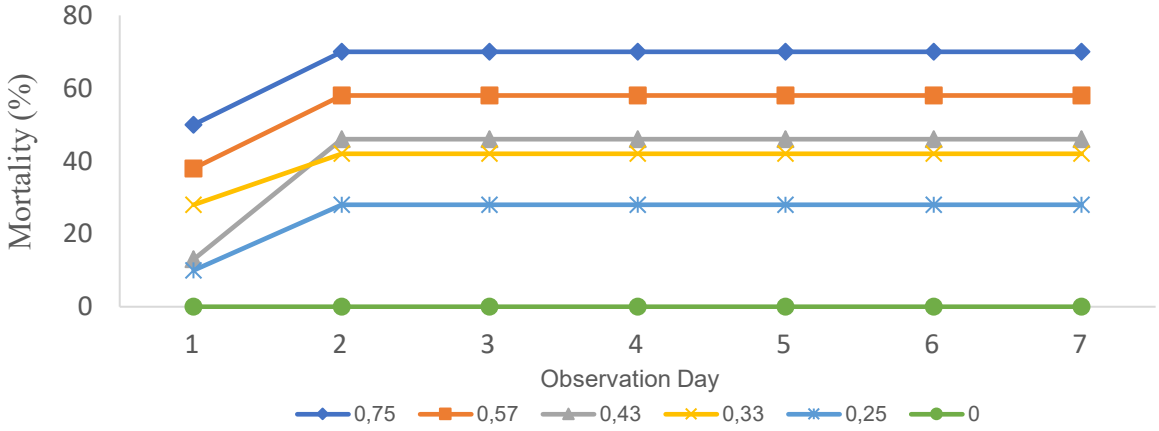


Figure 1. Development of *Spodoptera frugiperda* larval mortality after treatment in a nanoemulsion formulation of para Menthane-3,8-diol in a follow-up test

Probit analysis of PMD nanoemulsion formulations against *Spodoptera frugiperda* larvae revealed a regression slope of 2.24 ± 0.76, indicating a moderate dose–response relationship. The estimated lethal concentrations (LC_{25} and LC_{50}) of the PMD nanoemulsion were 0.15% and 0.48%, respectively (Table 2). These results demonstrate that the nanoemulsion formulation of PMD exhibited effective toxicity against *S. frugiperda* larvae at relatively low concentrations.

Table 2. Probit regression analysis of para-menthane-3,8-diol nanoemulsion against *Spodoptera frugiperda* larvae

Treatment	Nilai b ±SE	LC_{25}(%)	LC_{50}(%)
para-Menthane-3,8-diol nanoemulsion formulations	2.24±0.76	0.15	0.48

Antifeedant

The results of the PMD nanoemulsion formulation test on *Spodoptera frugiperda* showed an increase in the percentage of feeding inhibition with increasing concentration. The activity of the nano-emulsion formulation on the average leaf area consumed by *S. frugiperda* larvae treated with the PMD-based nanoemulsion showed a significant reduction compared to the control, indicating a higher level of antifeedant activity. At a concentration of 0.75%, the PMD nanoemulsion formulation was able to inhibit larval feeding by up to 62.88% (Table 3).

Pupae and adult formed

The study on the effects of applying the PMD nanoemulsion formulation to pupae and adult stages of *S. frugiperda* showed a tendency toward a decrease in the number of pupae and adult formed with increasing concentrations of the applied nanoemulsion. The activity of the PMD-based nanoemulsion formulation increased to 70% at the highest concentration (0.75%), which was not significantly different from the activity observed at a concentration of 0.57% (Table 4).

Table 3. Antifeedant effect of *Spodoptera frugiperda* larvae after treated with *para-Menthane-3,8-diol* nanoemulsion formulation

Concentration (%)	Leaf area consumed (mm^2)	Antifeedant (%)
0.00	899.54a	-
0.25	861.66ab	7.30
0.33	734.35 bc	18.36
0.43	652.23 cd	27.49
0.57	526.36 d	41.48
0.75	333.90 e	62.88

Notes: Numbers followed by the same lowercase letter in the same column are not significantly different based on LSD test at the 5% level

Table 4. *Spodoptera frugiperda* pupae and adult formed after treatment with para-menthane-3,8-diol nanoemulsion formulation

Concentration (%)	Pupae formed (%) ± SD	Adult formed (%) ± SD
0.00	100.00± 0.00 a	100.00± 0.00 a
0.25	72.00± 0.83 bc	72.00± 0.83 bc
0.33	58.00± 0.44 cde	58.00± 0.44 cde
0.43	54.00± 1.81 def	54.00± 1.81 def
0.57	42.00± 0.83 fgh	42.00± 0.83 fgh
0.75	30.00±0.70 h	30.00± 0.70 h

Numbers followed by the same lowercase letter in the same column are not significantly different based on LSD test results at the 5% level.

Sex ratio

The effect of adding an antifoaming agent to the PMD nanoemulsion formulation on the sex ratio of *S. frugiperda* showed a clear concentration-dependent reduction in the proportion of females. The most pronounced effect was observed at a concentration of 0.75%, where the sex ratio reached 1:0.22, representing the lowest female proportion among all treatments (Table 5).

Table 5. Sex rasio effect of *Spodoptera frugiperda* larvae after treated with *para-Menthane-3,8-diol* nanoemulsion formulation

Concentration (%)	Male: Female
0.00	1:2.33
0.25	1:1.76
0.33	1:1.07
0.43	1:0.68
0.57	1:0.26
0.75	1:0.22

Food Assimilation

Food assimilation analysis of *Spodoptera frugiperda* larvae showed that PMD nanoemulsion formulations at LC_{25} and LC_{50} concentrations resulted in significantly different growth rate, relative growth rate, and larval digestibility compared to the control. In contrast, the relative consumption rate, efficiency of food consumption, and efficiency of food digestion did not differ significantly from those of the control. Observations of growth rate and relative growth rate in the control treatment were higher than those observed in larvae treated with PMD nanoemulsion at LC_{25} and LC_{50}. Furthermore, the LC_{50} treatment showed a significantly different effect on growth rate compared to the LC_{25} treatment (Table 6).

Table 6. Food assimilation parameters of *Spodoptera frugiperda* larvae after treatment with para-menthane-3,8-diol (PMD) nanoemulsion formulation

Concentration (%)	Consumption rate (mg/day)[1)]		Growth rate (mg/day)[1)]		Food utilization efficiency (%)[1)]		
	CR	RCR	GR	RGR	DC	EFC	EFD
Control	0.0716 c	4.2824 a	0.0068 c	0.3336 b	0.0092 a	5.1971 a	5.6942 a
LC_{25}	0.0610 bc	4.3345 a	0.0030 ab	0.1003 a	0.0097 b	5.8229 a	6.0629 a
LC_{50}	0.0328 a	3.2440 a	0.0023 a	0.1181 a	0.0096 b	6.0029 a	6.1941 a

[1)]Numbers followed by the same letter are not significantly different (Duncan test at 5% level). CR (consumption rate), RCR (relative consumption rate), GR (growth rate), RGR (relative growth rate), DC (digestibility), EFC (efficiency of food consumed), EFD (efficiency of food digested).

Discussion

The PMD nanoemulsion formulation exhibited mortality, antifeedant activity and affected pupation rate, adult emergence, sex ratio, and food assimilation in *S. frugiperda* larvae (Table 1-6) Insects that survive insecticide exposure may still experience pronounced sublethal effects. Bartling et al. (2024) reported that sublethal exposure can alter insect physiology and behavior, including changes in locomotor activity. Similarly, sublethal concentrations have been shown to affect biological parameters such as reduced fecundity, prolonged developmental duration, and changes in pupal and adult body weight (Siahaya, 2021). Sublethal effects may interfere with morphological and physiological processes, including feeding inhibition, sex ratio, and food assimilation efficiency.

However, these effects did not significantly disrupt pupation or adult emergence. This outcome may be attributed to the availability of sufficient food and nutrients that allowed larvae to complete their development. In line with Erlina et al. (2020), insects consuming nutritionally adequate diets are able to maintain the continuity and quality of their developmental cycle.

The antifeedant activity of the PMD nanoemulsion against *S. frugiperda* increased proportionally with treatment concentration, as indicated by reduced leaf area consumption at higher concentrations (Table 2). This finding

is consistent with Husnun et al. (2022), who reported that citronellal compounds derived from lemongrass exhibit repellent properties against insects. Similarly, secondary metabolites from citronella were shown to reduce feeding activity of *Setora nitens* by 84.73% (Sutrisno, 2022). Although larvae continued to feed on treated leaves, consumption was minimal, particularly at higher concentrations, and feeding ceased after a short period. This pattern indicated a secondary antifeedant effect. According to Lina et al. (2015), plant-derived antifeedants may act through sensory detection mechanisms (primary antifeedant effects) or by disrupting neural regulation of feeding behavior after ingestion (secondary antifeedant effects).

The results indicate that the active compound paramethandiol primarily exhibits toxic effects at the larval stage and does not significantly affect metamorphosis. Larvae that survived exposure were still able to develop normally, resulting in pupation and adult emergence of Spodoptera frugiperda that were not significantly different from the control (Table 4). These findings suggest that PMD nanoemulsion does not interfere with hormonal regulation involved in molting and metamorphosis, such as ecdysteroid and juvenile hormones (Putro et al. 2016). The main mode of action of PMD nanoemulsion is therefore likely targeted at the larval stage through acute toxicity or antifeedant activity, when feeding behavior and metabolic activity are most intensive. Once larvae enter the pupal stage, feeding ceases and physiological stability increases, reducing sensitivity to the compound and limiting observable toxic effects.

The PMD nanoemulsion significantly influenced the sex ratio of *S. frugiperda*, with higher concentrations reducing the proportion of females (Table 5). This effect is likely associated with suboptimal nutrition intake or metabolic disruption caused by feeding on treated leaves, which may influence physiological mechanisms involved in sex determination. Under normal conditions, insect populations typically exhibit a balanced sex ratio; however, under stressful or unfavorable conditions, male-biased populations are more frequently observed. Stressful environments and exposure to toxic compounds have been reported to interfere with sex chromosome systems and reproductive physiology, resulting in reduced female emergence. Therefore, the PMD nanoemulsion formulation not only affected larval mortality but also altered population structure by shifting the sex ratio.

Food assimilation analysis revealed that growth rate, relative growth rate, and larval digestibility differed significantly between PMD nanoemulsion treatments and the control (Table 6). These differences reflected metabolic disruption caused by toxic compounds entering the larval body. Lina et al. (2021) reported that toxin accumulation increases metabolic demands and requires substantial energy allocation for detoxification processes. Larvae appear to compensate for these toxic effects by increasing digestibility, which enhances nutrient absorption efficiency (Lina et al., 2015). Although the efficiency of ingested food (ECI) and efficiency of digested food (ECD) increased at LC_{50}, these changes were not statistically significant compared with the control. Digestibility values were consistently higher in treated larvae,

suggesting increased food retention in the digestive tract.

Based on researches, insects feeding on suboptimal or toxic diets may increase digestibility as an adaptive strategy to optimize nutrient utilization (Wang et al., 2021; Chris-Ribeiro et al., 2025; Chamani et al., 2025). Alternatively, insects may increase consumption rate; however, rapid food processing reduces retention time in the gut, potentially lowering both consumption efficiency and digestion efficiency (Holtof et al., 2019). In the present study, increased digestibility accompanied by non-significant changes in ECI and ECD indicates a physiological adaptation strategy aimed at maintaining survival under toxic stress, consistent with the findings of Lina et al. (2021).

Conclusion

The results showed that Para-Menthane-3,8-Diol (PMD) nanoemulsion induced significant physiological interference in *S. frugiperda*, as indicated by increased mortality with LC_{25} and LC_{50} values of 0.15% and 0.48%, respectively. At a concentration of 0.75%, the treatment disrupted development by reducing pupation and adult emergence to 30% and altering the sex ratio to 1 male: 0.22 female. Sublethal interference was evident through strong antifeedant activity (62.88%) and pronounced changes in physiological parameters, including consumption rate (0.0328 mg/day), growth rate (0.0023 mg/day), and efficiency of ingested food (6.0029 mg/day). Overall, the PMD nanoemulsion formulation interfered with survival, feeding behavior, growth, and developmental physiology of *S. frugiperda*, highlighting its potential as an eco-friendly botanical insecticide.

Declaration

Author contribution

Rayhan Fadhlurrahman is the main contributor of this paper, Eka Candra Lina is the corresponding author, and Novri Nelly is co-author. All authors read and approved the final paper.

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Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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