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Toxicity and Developmental Disruption of *Piper aduncum* Nanoemulsion against *Paracoccus marginatus* under Laboratory Conditions

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Abstract

Paracoccus marginatus is one of the important pests that can reduce papaya production. *Piper aduncum* nanoemulsion has potential as a botanical pesticide to control papaya mealybug (*Paracoccus marginatus*). This present research was aimed to determine the most effective concentration in suppressing the development of *P. marginatus*. This study consisted of 6 treatments and 5 replications: 0.33%, 0.43%, 0.57%, 0.75%, 0.99%, and control. The observed parameters were: nymph mortality, adult mortality, daily mortality, adult emergence, LC_{50}, and LC_{95}. The data obtained were analyzed using variance (ANOVA) and 5% Tukey. The LC_{50}, and LC_{95} value was analyzed using Polo Plus. *Piper aduncum* nanoemulsion exhibits strong insecticidal activity against nymphs and adults of *Paracoccus marginatus* under laboratory conditions. Among the concentrations tested, 0.99% was the most effective, causing 81.11% nymph mortality at 72 hours post-application; adult emergence was limited to only 13.33%. The LC_{50} and LC_{95} values at 72 hours were 0.506% and 1.222%, reflecting the potency of the nanoemulsion at relatively low doses. Further research under field conditions is recommended to evaluate its efficacy, formulation stability, and safety on non-target organisms.

Keywords: *Mealybug*, botanical pesticide, mealybug, LC95, mortality rate

Introduction

Paracoccus marginatus (Hemiptera: Pseudococcidae) or papaya mealybug is a highly polyphagous and invasive pest that infests more than 200 plant species, many of which are economically important (Garcia et al., 2016). The pest causes significant damage by sucking sap from leaves, stems, and fruits, leading to wilting, yellowing, stunted growth, and even plant death (Maharani et al., 2016). In addition, it excretes honeydew that promotes the growth of sooty mold, reducing photosynthetic activity and overall plant vigor. Walker et al. (2003) highlighted its potential to cause widespread damage in newly invaded regions, posing serious threats to tropical and subtropical agriculture. Recent estimates in Africa reported an average crop loss of 57% due to *P. marginatus* infestations (Kansiime et al., 2023).

In Indonesia, *P. marginatus* is a relatively recent invasive pest and is considered difficult to control using synthetic insecticides due to the thick wax layer on its body, which limits pesticide penetration (Asnan et al., 2015).

Furthermore, excessive and repeated use of chemical insecticides has led to resistance development in *P. marginatus* populations (Mwanauta et al., 2021). The limited efficacy of chemical pesticides highlights the urgent need for alternative pest management strategies.

One promising approach is using botanical insecticides derived from plant extracts, which are considered more environmentally friendly, biodegradable, and safer for non-target organisms. However, studies exploring the use of botanical insecticides for *P. marginatus* management remain limited. This study selected *Piper aduncum* (Piperaceae) as a candidate botanical insecticide due to its well-documented insecticidal properties (Lina et al., 2025; Pebrulita, 2013). *P. aduncum* grows abundantly in the Sumatra region and contains secondary metabolites such as dillapiole (79.35%) that act as neurotoxins by blocking nerve impulse transmission in insect axons, leading to paralysis and death (Lina et al., 2015).

Previous studies have shown that *P. aduncum* fruit extract at 0.5% concentration can cause 100% mortality in *Crocidolomia pavonana* larvae, and has also demonstrated effectiveness against *Sitophilus oryzae* (Andrianto et al., 2017), armyworms on soybean (Agustina et al., 2017), and *Spodoptera frugiperda* (Lina et al., 2025). At 1% concentration, the extract caused 93% larval mortality within 72 hours after treatment. Despite these findings, studies on its efficacy against *P. marginatus* are still scarce.

To enhance the effectiveness of botanical insecticides, the integration of nanotechnology into pesticide formulation is gaining traction. Nanoparticles, typically ranging from 20 to 200 nm in size (El-Brolossy et al., 2008), offer several advantages over conventional formulations, including increased stability, enhanced penetration through insect cuticles, longer shelf life, and reduced environmental impact. Among various nano-formulations, nanoemulsions are particularly promising due to their small droplet size, ease of preparation, and improved bioavailability (Erlina et al., 2020; Kapinder et al., 2020).

A nanoemulsion formulated with *P. aduncum* fruit oil and *Cymbopogon nardus* distillate waste has shown strong insecticidal activity against *Spodoptera frugiperda*, with an LC_{50} value of 0.53% and demonstrated developmental inhibition (Lina et al., 2025). In addition, field application of nanopesticides has proven effective in reducing pest-associated diseases in chili, such as curly leaf and yellow virus (Ernita et al., 2021; Septariani et al., 2020).

Given the biological potential of *P. aduncum* and the advantages of nanoemulsion technology, this study aimed to evaluate the toxicity of *P. aduncum* nanoemulsion in controlling *P. marginatus* under laboratory conditions. This work is expected to contribute to developing sustainable pest management strategies and provide a safer alternative to synthetic insecticides.

Methods

The research was conducted at the Insect Bioecology Laboratory, Department of Plant Protection, Faculty of Agriculture, Andalas University, Padang from January 2024 to May 2024.

Experimental Design

The experiment followed a Completely Randomized Design (CRD) and was carried out in two phases: preliminary testing and advanced bioassays.

The preliminary test aimed to determine a concentration range of *Piper* *aduncum* nanoemulsion that could induce 10–90% mortality of *Paracoccus marginatus* nymphs. Four concentrations (0.25%, 0.5%, 0.75%, 0) were tested, each replicated six times. Each replicate consisted of 10 third-instar *P. marginatus* nymphs exposed to treated papaya leaves. Mortality was recorded at 24, 48, and 72 hours post-treatment.

Based on the preliminary test, six concentrations (0.33%, 0.43%, 0.57%, 0.75%, 0.99%, 0) were selected for further evaluation. Each treatment was replicated five times with 15 third-instar nymphs per replicate. Observations included cumulative mortality at three time intervals (24, 48, and 72 hours), daily mortality from day 1 to day 5, adult emergence, and lethal concentration estimates (LC_{50} and LC_{95}).

Rearing of *P. marginatus*

Adult of *P. marginatus* was collected from papaya fields at the Tropical Fruit Research Institute, West Sumatra, Indonesia. Colonies were maintained on four-week-old papaya seedlings grown in polybags. Three seedlings were placed in plastic cages (30 cm diameter × 15 cm height) covered with gauze. The insects were reared for three generations to ensure a healthy and synchronized population. Third-instar nymphs from the F3 generation were used for bioassays.

Extraction of *P. aduncum*

P. aduncum extraction was done using a maceration method using ethyl acetate solvent. In the first stage, *P. aduncum* leaves were cut into 1 cm pieces and air-dried without direct sunlight for 3 weeks. After drying, the material was blended until smooth and sieved until a fine powder was obtained. In the next stage, each powder was weighed at 50 g, put into a separate Erlenmeyer flask, and soaked in 500 mL of ethyl acetate solvent for 2 x 24 hours. Then the extracted liquid was filtered using a glass funnel (diameter 9 cm) lined with ordinary filter paper and collected in an Erlenmeyer flask. The first filter result was filtered again using a glass funnel (diameter 5 cm) based on Whatman No. 41 filter paper and collected in a steam flask. The second filter was evaporated with a rotary evaporator at a temperature of 50 ℃ and a pressure of 240 mbar. Ethyl acetate obtained from evaporation is used to re-soak the plant extract dregs up to 3 times, soaking (2 subsequent soakings for 1 x 24 hours). The extract obtained in the flask is a semi-solid material.

Nanoemulsion Formulation

A nanoemulsion was made with spontaneous emulsification, which occurs when the organic and water phases are mixed. The emulsion system consists of an organic phase (extract = *P. aduncum*, solvent = ethanol) and a water phase (water = distilled water and surfactant = Tween 80). The organic phase was made by mixing *P. aduncum extract* and solvent, as much as 10% of the total emulsion, with a composition of 1:1 (w/v). The water phase was made by mixing 3% Tween 80 and distilled water, stirring using a magnetic stirrer for 30 minutes at room temperature. The spontaneous emulsification technique was carried out by adding the organic phase to the water phase by dropping (drop by drop) and continued with magnetic stirring for 45 minutes (Lina et al., 2021).

Bioassay Procedure

The preliminary test of *P. aduncum* was conducted at control concentrations: 0.25, 0.5, and 0.75% with 6 replications each. Each replication was filled with 10 *P. marginatus instar* III nymphs. Young and fresh papaya leaves free of insecticide were cut into pieces measuring 4 cm x 4 cm. The leaves were dipped for 1 minute, then air-dried. One piece of treatment leaf and one control leaf were placed separately in a petri dish (with a diameter of 9 cm). Instar III nymphs were placed in each petri dish containing treatment leaves/control leaves. Dead nymphs were counted and discarded from the petri dish. Observations were made at 24, 48, and 72 hours after application. Further tests used concentrations of 0.33%, 0.43%, 0.57%, 0.75%, 0.99%, and a control with 5 replications each. Each replication was filled with 15 *P. marginatus* nymphs. *P. marginatus* instar III. The implementation of the follow-up test is the same as the preliminary test.

Observation

Nymph mortality (%)

Mortality of *P. marginatus* nymph was observed every 24 hours after application. Observations were made by calculating larval mortality using the formula:

$$M=\frac{n}{N}\ x\ 100\% \quad (1)$$

Notes: M = Larval mortality (%), n = Number of dead larvae, N = Number of larvae treated

Daily mortality

Dead *P. marginatus* nymphs were recorded and observed from day 1 to day 5 after treatment.

Adult emergence

The emergence adults are counted and recorded, including how many female adults aemergence, male adults, the total number of all adults, and the sex ratio between male and female adults. The percentage of successful adult formation can be calculated using the formula:

$$M=\frac{\Sigma\ Adults\ are\ emerging}{\Sigma\ number\ of\ nymphs\ tested}\ x\ 100\% \quad (2)$$

Lethal concentration (LC_{50} and LC_{95})

Lethal concentration values (LC_{50} and LC_{95}) were determined using probit analysis with the POLO Plus software. These values represent the concentrations required to kill 50% and 95% of the treated population, respectively. Lower LC values indicate higher insecticidal potency and effectiveness of the *Piper aduncum* nanoemulsion against *P. marginatus*.

Data Analysis

All experimental data, including mortality percentages, adult emergence rates, and LC values, were analyzed using one-way analysis of variance (ANOVA). Significant differences among treatments were further evaluated using Tukey's test at a 5% level of significance.

Results

Preliminary Test

Nymph mortality (%)

The results showed that *P. aduncum* nanoemulsion affected the mortality of *P. marginatus* instar III. The highest mortality was found in the treatment of *P. aduncum* nanoemulsion concentration of 0.75% with nymph mortality of 80% after 72 hours after treatment (P = 0.00). The concentration of 0.75% can kill *P. marginatus* nymphs significantly different from all treatments at 24 hours, 48 hours, and 72 hours after application of *P. aduncum* nanoemulsion to *P. marginatus* larvae (Table 1).

The results of preliminary test observations provide an overview that all concentrations of *P. aduncum* nanoemulsion can kill *P. marginatus* nymphs ranging from 10% to 90% which are used as initial concentrations for further tests.

Advanced Test

Nymph mortality (%)

The results of the study showed that *P. aduncum* nanoemulsion affected the mortality of *P. marginatus* instar III nymphs. The highest mortality was found in the treatment of P. *aduncum* nanoemulsion concentration of 0.75% with nymph mortality of 81.11% (Table 2, Figure 1).

Table 1. Preliminary test of *P. marginatus* nymph mortality with *P. aduncum* nanoemulsion application (%).

Treatment (%)	Mortality of *P. marginatus* nymphs (%) ± Standard deviation											
	24 hours				48 hours				72 hours			
0.75	60.00	±	0.52	a	68.33	±	0.98	a	80.00	±	0.63	a
0.5	43.33	±	0.63	b	50.00	±	0.89	b	51.67	±	0.41	b
0.25	11.67	±	0.41	c	20.00	±	0.52	c	23.33	±	0.63	c
Control	0.00	±	0.00	d	0.00	±	0.00	d	0.00	±	0.00	d

*Numbers in the same column followed by different letters indicate significant differences based on a tukey (5%)

Table 2. Advanced test of *P. marginatus* nymph mortality with application of *P. aduncum* nanoemulsion (%).

Treatment	Mortality of *P. marginatus* nymphs (%) ± Standard deviation											
	24 hours				48 hours				72 hours			
Control	0.00	±	0.00	d	0.00	±	0.00	d	0.00	±	0.00	e
0.33	22.20	±	0.52	c	28.89	±	0.52	c	34.45	±	0.75	d
0.43	30.00	±	0.55	c	34.45	±	0.75	c	41.12	±	0.75	d
0.57	38.89	±	0.75	b	43.33	±	0.55	b	58.89	±	0.41	c
0.75	44.44	±	0.52	b	53.33	±	0.63	a	70.00	±	0.55	b
0.99	53.33	±	1.10	a	60.00	±	1.10	a	81.11	±	0.75	a

*Numbers in the same column followed by different letters indicate significant differences based on a tukey (5%)

Figure 1. Nymph of *Paracoccus marginatus*: (a) treated with *P. aduncum* nanoemulsion (b) normal

Daily Mortality (%)

The results of observations on the percentage of daily mortality of *P. marginatus* nymphs with different concentrations of *P. aduncum* nanoemulsion treatments showed an effect on the death of *P. marginatus* nymphs. Mortality increased along with the observation time, with the highest being on the 5th day. The concentration in each observation also showed that 0.99% had high mortality in *P. marginatus* (Figure 2).

The most effective concentration of application of *P. aduncum* nanoemulsion was 0.99 %. Observation of daily mortality of nymphs *P. marginatus* showed an increase in mortality from day 1 to day 5 after applying *P. aduncum* nanoemulsion (Effectiveness 34.48%- 83.90%) (Table 3).

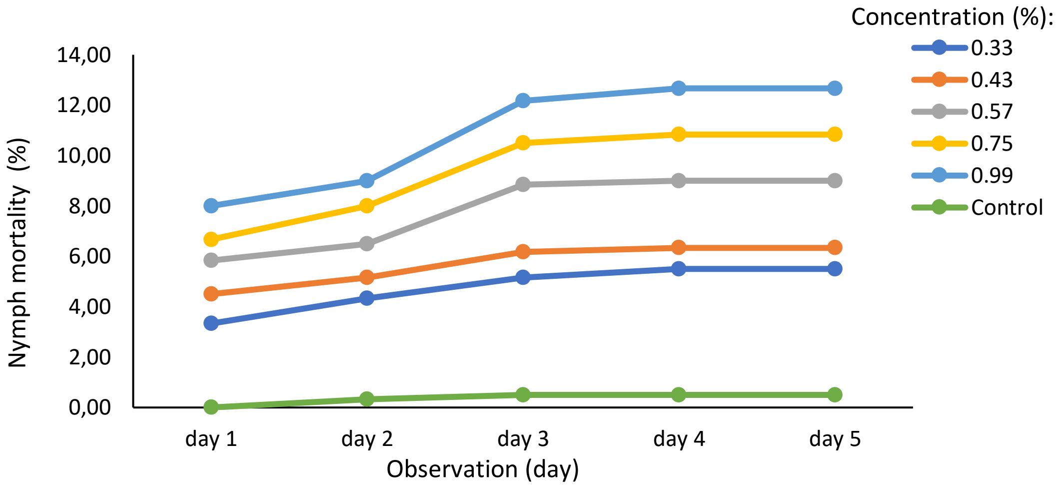

Figure 2. Daily mortality of *P. marginatus* nymphs after application of *P. aduncum* nanoemulsion

Table 3. Effectiveness of *P. marginatus* nymphs after application of *P. aduncum* nanoemulsion

Treatment	Effectiveness (%)
Control	0.00
0.33	34.48
0.43	40.23
0.57	58.62
0.75	71.26
0.99	83.90

Adult Emergence

The results of the maintenance of nymphs treated with *P. aduncum* nanoemulsion affected the number of adults emergence. The number of *P. marginatus* adult nymphs became low after the application of *P. aduncum* nanoemulsion. The concentration that affected the adults in the 0.99% concentration treatment, with a total percentage of adult emergence, was 13.33% lower than that of other treatments. Adult emergence were more female adults compared to male adults with a ratio of 1:3 (Table 4).

Lethal Concentration (LC_{50} and LC_{95}) (%)

Based on the value of the probit lethal concentration (LC) analysis results which are a measure of the toxicity of a material, *P. aduncum* nanoemulsion is effective *against P. marginatus* nymphs with LC_{50} and LC_{95}, which are 0.509 and 1.22, respectively (Table 5)

Table 4. Number of *Paracoccus marginatus* adult emergence after application of *Piper aduncum* nanoemulsion (%)

Treatment	Adults emergence (%) ± Standard deviation		
	Male	Female	Total adults
Control	28.89 ± 0.82 a	64.44 ± 0.82 a	93.33 ± 0.63 a
0.33	14.44 ± 5.02 b	48.89 ± 0.82 b	63.33 ± 0.55 b
0.43	12.22 ± 9.81 bc	44.44 ± 0.82 b	56.67 ± 1.38 b
0.57	11.11 ± 3.44 bc	26.67 ± 0.63 c	37.78 ± 0.52 c
0.75	4.44 ± 3.44 cd	20.00 ± 0.63 cd	24.44 ± 0.52 d
0.99	1.11 ± 2.72 d	12.22 ± 0.41 d	13.33 ± 0.63 e

*Numbers in the same column followed by different letters indicate significant differences based on a tukey (5%)

Table 5. Lethal concentration (LC_{50} and LC_{95}) values of *P. aduncum* nanoemulsion against *P. marginatus* nymphs

Treatment	LC_{50}	LC_{95}
24 hours	0.814	1.950
48 hours	0.690	1.764
72 hours	0.506	1.222

*Lower LC_{50} and LC_{95} values indicate higher toxicity of the nanoemulsion, as less concentration is needed to cause mortality in *P. marginatus* nymphs.

Discussion

P. aduncum nanoemulsion with several different concentrations can suppress the development of *P. marginatus*. *P. marginatus* can kill nymphs because it contains several compounds, such as hexane, cyanide, saponins, tannins, flavonoids, steroids, alkaloids, and essential oils, which are thought to function as botanical pesticides. Forest betel leaf essential oil contains 43.3% dilapiol, 8.2% β-caryophyllene, 6.7% piperitone, 5.1% α-humulene, and other compounds, each less than 5% (Rali et al., 2007). Mortality of nymphs in the preliminary test showed that all concentrations tested could kill *P. marginatus* nymphs (Table 1). This concentration is a reference in determining the concentration of further tests. The ability of *P. aduncum* nanoemulsion to kill nymphs is influenced by the concentration and observation time interval of *P. marginatus* nymph deaths after application. High concentrations can kill nymphs more effectively in suppressing the development of *P. marginatus* (Table 2).

Mortality of nymphs increases with increasing concentration levels given. This is because the amount of active ingredient content differs at each concentration level. The difference in concentration in killing nymphs is due to the amount of active ingredient content that enters the insect's body being greater and strong enough to kill insects (Lina et al. 2015). The active ingredients in *P. aduncum* have a lethal effect on *P. marginatus*. The main impact of *P. aduncum* nanoemulsion on *P. marginatus* nymphs is disruption of the insect's nervous and respiratory systems, which causes paralysis and, ultimately, death. This mechanism is caused by phenolic and monoterpene compounds inhibiting necessary insect enzymes (Yactayo-Chang et al., 2020). Applying *P. aduncum* nanoemulsion at a concentration of 1-2% can cause up to 90% mortality in nymphs within 72 hours after applying *P. aduncum* (Rodrigues et al., 2020).

Daily mortality of *P. marginatus* nymphs for five days increased (Figure 2). Mortality is usually low on the first to the second day because nymphs need time to be exposed to lethal doses of active compounds such as dillapiole and phenolics in *P. aduncum* nanoemulsions. Some of these compounds work actively by inhibiting enzymes such as acetylcholinesterase, one of which can interfere with the insect's nervous system (Rido et al., 2020). On the third to fifth day, there was a significant increase in mortality because the active compounds had reached their maximum accumulation levels in the nymph's body, causing metabolic disorders, respiration, and ultimately death (Rustam & Simarmata 2022). The compound content in *P. aduncum* nanoemulsions penetrates the insect's cuticle wax layer, increasing the absorption of active compounds and accelerating the toxic impact (Gupta et al., 2024).

P. aduncum nanoemulsion has great potential in suppressing the number of adults of *P. marginatus* through a control mechanism that affects the developmental stages of insects (Table 3). Active compounds in *P. aduncum* essential oil, such as dillapiole, phenol, and monoterpene, are known to have larvicidal and oviposition effects, which disrupt the life cycle of insects, including the development from nymphs to adults (Santos et al., 2017). In *P. marginatus* nymphs, applying *P. aduncum* nanoemulsion causes physiological disorders by effectively penetrating active compounds into the insect's body. This nanoemulsion damages the integrity of the insect cuticle, inhibits the respiratory system, and disrupts the insect's developmental hormone system, such as juvenile hormone and ecdysone, which play an important role in the molting and metamorphosis processes ((Yactayo-Chang et al., 2020). As a result, nymphs fail to develop into adults in optimal numbers. The decrease in the number of adults is also caused by the residual effect of the nanoemulsion that prevents nymphs from surviving during the developmental stages. A study by Rodrigues et al. (2020) showed that applying *P. aduncum* nanoemulsion at a concentration of 1% could suppress adult formation by up to 85% compared to the control. This effect occurs because the active compounds in the nanoemulsion formulation are toxic and have antifeedant and repellent activities, which reduce nutrient consumption by nymphs, thereby slowing or stopping their development.

Determination of LC_{50} and LC_{95} values is an important step to evaluate the effectiveness of insecticides, including *P. aduncum* nanoemulsion against *P. marginatus* nymphs. *P. aduncum* nanoemulsion has low LC_{50} and LC95 values, around 0.506 - 1.222% against *P. marginatus* nymphs within 72 hours after application. The low LC50 and LC95 values indicate that active compounds such as dillapiole and monoterpenes in *P. aduncum* essential oil are very toxic to target insects (Table 4).

A low-dose (≤1%) nanoemulsion formulation of *Piper aduncum* is acutely toxic to *P. marginatus* nymphs and disrupts their development and adult emergence, providing a multi-target mechanism of action. Unlike synthetic insecticides, which typically act on a single target site, this botanical nanoemulsion shows promise for integrated pest management (IPM) strategies with reduced environmental risk and lower potential for resistance development.

Conclusion

Piper aduncum nanoemulsion exhibits strong insecticidal activity against nymphs and adults of *Paracoccus marginatus* under laboratory conditions. Among the concentrations tested, 0.99% was the most effective, causing 81.11% nymph mortality at 72 hours post-application; adult emergence was limited to only 13.33%. The LC_{50} and LC_{95} values at 72 hours were 0.506% and 1.222%, reflecting the potency of the nanoemulsion at relatively low doses. Further research under field conditions is recommended to evaluate its efficacy, formulation stability, and safety on non-target organisms.

Declaration

Author contribution

Mega Andini is the main contributor for this paper. Eka Candra Lina is corresponding author. 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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