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Bioactivity Test of Gambir (*Uncaria gambir* Roxb) Processing Waste as an Environmentally Friendly Alternative for Pest Control Using Nano Technology

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ABSTRACT

The gambir plant can serve as a raw material for botanical insecticides. Plant-based insecticides are made in nanoemulsion form to overcome the insecticide particle size problem. This study aims to obtain a gambir waste form that has the potential to be used as a botanical insecticide using nanotechnology. The laboratory experiments include preliminary and follow-up tests. The preliminary test involves testing each extract (liquid, solid, and raw gambir waste) at three concentrations using a completely randomized design with four replications. The follow-up test uses the residue method on leaves to test insecticidal activity. Creating nanoemulsion from gambir waste involves mixing organic and liquid phases through spontaneous emulsification. The insecticidal effectiveness of this nanoemulsion is tested on *Croccidolomia pavonana*. The results show that solid and liquid gambir waste has the potential to be used as alternative insecticides, which can influence the mortality of *C. pavonana* with an LC_{50} concentration of 0.22 (solid waste) and 0. 29 (liquid waste), while LC_{95} is 2.44 (solid waste) and 2.52 (liquid waste). The research promotes utilizing natural resources and innovative technologies, advancing environmentally conscious pest control methods, and fostering sustainable agricultural systems.

INTRODUCTION

Farmers use synthetic pesticides to control pests. The continuous use of synthetic pesticides will harm the environment and surrounding living creatures. Chowański et al. (2014) state that although synthetic insecticides are efficient for insect control, they carry environmental and health risks. They disrupt ecosystem functions, are toxic to various non-target organisms, and tend to accumulate in the environment. Samanta et al. (2023) argue that the use of synthetic pesticides in agricultural areas poses a threat to both natural predators and pollinators. Therefore, developing eco-friendly pesticide alternatives and integrated pest management techniques is desirable to reduce the impacts of pesticides (Kim et al., 2017). One control that is safe and friendly to the environment is using natural materials as raw materials for making botanical pesticides. According to Haritha et al. (2021), botanical pesticides are alternatives to synthetic chemical pesticides for insect and pest control in agriculture. Botanical pesticides have advantages, such as leaving no residues in the plant system, and are not only biodegradable but also easily physically degradable, like temperature and ultraviolet.

The need to provide insecticide products derived from plants has encouraged various research into plant materials that can be a source of insecticides. Gambir (*Uncaria gambir* Roxb) is one of the plants that can be used as a raw material for botanical insecticides. The gambir plant contains various chemical compounds such as catechine,

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catechu tannin acid, pyrocatechol, quercitin, fluorescence, oil, wax, and alkaloids (Arumsari, 2021). The research of Dibisono et al. (2022) shows that gambier leaf extract at 32% concentration could cause the *Sethotosea asigna* death rate to reach 100%, while the intensity of attacks on the leaves decreased by 14.28%. Idris (2015) also found that leaf extract gambir has insecticidal properties and can better influence the physiological properties and hormones of insects *Plutella xylostella*. Apart from gambir leaves, gambir waste also has the potential to be a botanical insecticide. Kasim et al. (2019) state that gambir waste contains compounds such as pyrocatechol and phloroglucinol, which can potentially be used as waste from gambier processing.

Several weaknesses are found in making plant-based pesticide formations. Lina et al. (2017) state that several weaknesses found include the number of raw materials needed is still high, and the formulation can only protect the surface of the leaves, so it cannot be used to control pests in plant tissue or insects that have piercing-sucking mouthparts. Therefore, technology that can reduce these weaknesses is needed. One technology that can be used is nanoparticle technology. Nanoparticles are characterized as particles with sizes of 20-500 nm (Prabha et al., 2016). Nanoparticles currently being developed in pesticides are formulations in the form of nanoemulsions. The active ingredients can quickly enter the insect's body due to the size of the nanoparticle (Erlina et al., 2020). Nanoemulsions containing a mixture of *Tephrosia vogelii* and *Piper aduncum* extracts show synergistic effects. The activity of the extract mixture is higher than the individual extracts against *Crocidolomia pavonana* larvae in cabbage (Lina et al., 2021). This study aims to obtain a gambir waste form that has the potential to be used as a botanical insecticide using nanotechnology. Utilizing gambier processing waste using nanotechnology to obtain plant-based insecticides is one way to utilize waste and obtain plant-based insecticides that can control environmentally friendly pests.

MATERIALS AND METHODS

The research was conducted in the Insect Bioecology Laboratory, Plant Protection Department, Faculty of Agriculture, Universitas Andalas, from June to October 2023. The study involved both preliminary and follow-up tests. In the preliminary test, each extract (liquid, solid, and raw gambir waste) was tested at a concentration of 0.0%, 0.1%, and 0.5%. Each extract was tested individually by adding citronella hydrosol. The test used a completely randomized design with four replications. The follow-up test used the chosen extract and used the residue method on leaves to test insecticidal activity. The manufacture of botanical insecticides in nanoemulsions with active ingredients from gambir waste consists of the organic and the liquid phases. The two phases were mixed using a spontaneous emulsification method. Nanoemulsion was tested on *C. pavonana* to see the insecticidal activity of the nanoemulsion.

Provision of Liquid Waste, Solid Waste, and Raw Gambir

Liquid, solid, and raw gambir waste were obtained from the gambir processing industry center in Payakumbuh, West Sumatra. Gambir waste was filtered to remove dirt carried along with the waste using a filter cloth, followed by 100 mesh filtration to ensure that all fine dirt could be filtered. The filtered filtrate was used for treatment. Thickened waste raw materials were provided by heating gambir waste at 60-70℃. Heating above 75℃ caused the waste color to change to dark.

Solid waste was sediment from liquid waste obtained from the same gambir processing site in Payakumbuh, West Sumatra. Raw gambir waste was also obtained from the same place as a positive comparison.

Making Nanoemulsion

Nanoemulsion was prepared using a spontaneous emulsification mechanism and then tested using a Particle Size Analyzer (PSA) tool. Nanoemulsion with spontaneous emulsification is a nanoemulsion that occurs when the organic and water phases are mixed. The emulsion system consists of an organic phase (liquid waste or solid waste gambir and bioethanol) with a ratio of 1:1 and a water phase (distilled water and surfactant (Tween 80) with a ratio of 87:3. The organic phase was made by mixing gambir waste and 10% of the total nanoemulsion solution with a composition of 1:1 then stirring using a magnetic stirrer for 30 minutes. The water phase was made by mixing 3% surfactant (Tween 80) and 87% gambir boiled waste and stirring using a magnetic stirrer for 30 minutes at room temperature. The spontaneous emulsification technique was carried out by adding an organic phase of 10% of the total nanoemulsion solution, which was made into an aqueous phase by dripping and followed by stirring for 45 minutes. The composition of botanical insecticide nanoemulsion made from gambir waste was followed by a bioassay to obtain the composition with the highest activity. The test was carried out using the residue method on leaves.

Food Preparation of Insect

Broccoli seeds were sown in seedling trays filled with soil and compost. After the seedlings were four weeks old or had four leaves, they were transferred to a 10 kg polybag containing a mixture of soil and manure in a ratio of 3:1. Each polybag was filled with one broccoli plant seed. For maintenance, NPK fertilizer was given at a dose of ± 1 g per polybag. Next, maintenance starts with watering, weeding, and mechanical pest control. Once the broccoli plants were two months old, the leaves could be used as food for *C. pavonana* larvae.

Rearing of Test Insects

Croccidolomia pavonana larvae were collected from cabbage plantations in the Pandai Sikek area, Tanah Datar Regency. The larvae were taken to the laboratory and kept in plastic boxes (12 cm in diameter and 7 cm in height) with a hole in the lid and gauze. Broccoli leaves were put in a plastic box as food for *C. pavonana* larvae. Every day, the larvae's food was changed to maintain their health. Larvae that have turned into pupae were collected and placed in insect cages (30 cm x 30 cm x 30 cm). After the imago emerged, a film bottle filled with water and broccoli leaves was put in a cage as a place to lay eggs and given food in the form of liquid honey. The purpose of providing water in film bottles was to maintain the freshness of broccoli leaves for laying eggs. The larvae from the hatched eggs were transferred into plastic boxes containing broccoli leaves. The larvae could be test insects in the second instar (Lina et al., 2021). The test insects were third-generation because they were free from synthetic insecticide residues.

Preliminary Test

Each extract was tested at a concentration of 0.0, 0.1, and 0.5% with four repetitions, and in each repetition, second instar larvae were used. The extract was dissolved in acetone-methanol (3:1). The extract solution was mixed with citronella distillation waste and then applied to both sides of the leaf surface (3 cm diameter) at 25 µl/side using a microsyringe. Control (0.0%) leaves were treated with solvent alone in the same manner and volume. After drying, two leaves were put into a petri dish (9 cm diameter) lined with filter paper, and then the second instar larvae were put into it. The larvae were allowed to feed on the treated leaves for 48 hours; then, the leaves were replaced with fresh, pesticide-free leaves.

Follow-up Test

Follow-up tests were carried out to obtain LC_{50} and LC_{95} values. The experimental unit consisted of a petridish containing 15-second instar *C. pavonana* larvae fed broccoli leaves. Broccoli leaves (4 x 4 cm^2) were dipped in each treatment for 2 minutes. Then, they were air-dried and placed in a petridish (lined with tissue) containing the test larvae. Application of treatment leaves was carried out 2 x 24 hours. In the next 24 hours, the feed was replaced with untreated broccoli leaves. Mortality observations were carried out every day, starting 24 hours after treatment was given.

Observation

Living larvae were maintained until the fourth instar, and dead larvae were recorded daily. The development of living larvae was observed daily, and development time was calculated until they reached the fourth instar. The Abbot formula corrected larval mortality in the treatment for control mortality.

Analysis Data

The data analysis by ANOVA and the Least Significant Difference (LSD) test (α = 0.05) was conducted in case of significant differences. Statistical analysis of raw data and calculations used Microsoft Excel, while the following analysis used Statistix 8 software. Probit analysis was used for bioassay testing the relationship between solid and liquid gambir waste nanoemulsion concentration and citronella hydrosol on the mortality of *C. pavonana* larvae.

RESULTS AND DISCUSSION

Larval Mortality in Preliminary Tests

The results of preliminary tests on the mortality of *C. pavonana* larvae showed that the mortality after treatment with solid waste, liquid waste, and raw gambir nanoemulsions significantly influenced the concentration. Generally, increased concentration led to a higher percentage of larval mortality. In the test using solid gambir waste nanoemulsion, the concentration of 0.50% showed the highest mortality at 29.99%, while at 0.10%, it exhibited a mortality of 13.33%. The mortality of *C. pavonana* larvae in the test with liquid gambir waste nanoemulsion indicated that the 0.50% and 0.10% concentrations had a significantly different effect compared to the control. The mortality rate at the 0.50% concentration was 19.99%, whereas at the 0.10% concentration, it was 14.99%. In the test with raw gambir nanoemulsion, the highest concentration of 0.50% had a significantly different effect than the control, resulting in a mortality rate of 14.99%.

In comparison, at the 0.10% concentration, insect mortality is 6.66% (Table 1). These results show that gambir waste has the potential to be an active ingredient in botanical pesticides for environmentally friendly alternative control of *C. pavonana***.** The test results on three different types of gambir waste show different mortality. The three gambir wastes contain compounds such as tannin, which could kill *C. pavonana* through a stomach poisoning mechanism. According to Tan et al. (2022) tannic acid can be used as a plant-derived pesticide. Aprely et al. (2021) further suggest that tannin from gambir has been widely used as an antibacterial or antidiabetic. Tannin is a complex organic compound that is a secondary metabolic product of higher plants that bind proteins so that protease enzymes cannot degrade proteins (Setyorini & Antarlina, 2022). It prevents excess nitrogen, which causes plants to become dark green and wilt, making plants more susceptible to pests and disease. Sun et al. (2020) state that excess nitrogen can increase pest and disease attacks.

The preliminary test on *C. pavonana* larval mortality, after treatment with solid gambir waste nanoemulsion and lemongrass hydrosol, showed significant differences between the extract concentration and the control (without extract). The highest concentration at 0.50% exhibited a noteworthy impact, resulting in a mortality rate of 74.99%, followed by 0,01% with 53.33%. Similarly, the mortality of *C. pavonana* larvae after treatment with liquid gambir waste nanoemulsion and lemongrass hydrosol showed the difference between extract concentration and control. The highest concentration at 0.50% demonstrated a significant impact, leading to a mortality rate of 68.33%, while the 0.01% concentration was 38.33%. Additionally, the mortality of *C. pavonana* larvae in treatment with raw gambir nanoemulsion showed a significant difference between each concentration and the control, although the mortality value did not reach 50%. The highest concentration at 0.50% displays a considerable impact, resulting in a mortality rate of 24.55%, while 0.01% at 18.33% exhibits an increasing trend in larval mortality with higher treatment concentrations (Table 2).

Table 1. Mortality of *Crocidolomia pavonana* larvae after being treated with nanoemulsion of solid, liquid, and raw gambir waste during the preliminary test

Concentration (%)	**Mortality (%) ± SD**		
	Solid gambir waste	**Liquid gambir waste**	**Raw Gambir**
0.50	29.99 ± 3.85 a	19.99 ± 5.44 a	14.99 ± 3.33 a
0.10	13.33 ± 9.42 a	14.99 ± 10.00 a	6.66 ± 0.00 a
0.00 (Control)	1.66 ± 6.66 b	1.66 ± 0.00 b	1.66 ± 6.66 b

Remarks: Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at P<0.05

Table 2. Mortality of *Crocidolomia pavonana* larvae after being treated with nanoemulsion of solid, liquid, and raw gambier waste with the addition of citronella hydrosol during the preliminary test

Concentration (%)	**Mortality (%) ± SD**		
	Solid gambir waste	**Liquid gambir waste**	**Raw gambir**
0.50	74.99 ± 6.38 a	68.33 ± 6.38 a	24.55 ± 8.38 a
0.10	53.33 ± 12.17 a	38.33 ± 8.38 b	18.33 ± 8.38 a
0.00 (Control)	0.00 ± 0.00 b	0.00 ± 0.00 c	0.00 ± 0.00 b

Remarks: Numbers followed by the same lowercase letter in the same column are not significantly different according to the LSD test at P<0.05

The results of the mortality test from the three gambir wastes show that adding hydrosol can increase the percentage of mortality when compared to without hydrosol because hydrosol is a water emulsion solution containing essential oil components. According to Vuko et al. (2021), hydrosols from various plants are gaining importance in cosmetology, aromatherapy, traditional pharmacy, the food industry, and plant protection. Lina et al. (2021) state that mixing active plant compounds can provide synergistic, antagonistic, or additive effects. In a plant extract, apart from several main active compounds, there are usually many other less active compounds, but their presence can increase the overall activity of the extract (synergy).

In addition, fewer active ingredients are used in nanoemulsion formulations. Mushtaq et al. (2023) stated that Nanoemulsion-based methods efficiently encapsulate active ingredients, including antioxidants, lipids, vitamins, and antimicrobial agents, owing to the small droplet size, stability, and improved biological activity. If the mortality percentages of the three gambir wastes were compared, solid gambir waste was better than liquid and raw gambir waste because solid gambir waste was formed from dried sap, so it had a higher tannin content. In contrast, raw gambir waste did not meet the effectiveness criteria. That is why this study did not continue with the next testing stage.

Larval Mortality in Follow-up Tests

This study investigates the efficacy of gambir waste nanoemulsion in controlling *C. pavonana* larvae, revealing notable concentration-dependent effects. Solid and liquid gambir waste treatments exhibit significant differences from the control, with increasing concentrations correlating proportionally with higher larval mortality rates (Table 1 and Table 2). The highest concentrations (0.75%) result in peak mortality, reaching 84.00% for solid treatment and 78.66% for liquid treatment. Even at the lowest concentrations (0.01%), mortality has a discernible impact: 30.66% for solid treatment and 20.00% for liquid treatment (Fig. 1 and Fig. 2). It indicates the sensitivity of *C. pavonana* larvae to the treatments. Temporal patterns show increased mortality from day one to day three, leveling off from the third to the sixth day (Fig. 1 and Fig. 2). Moreover, replacing treated leaves with untreated ones on the third day led to a decline in tannin content, causing physiological disturbances and influencing larval development. These findings underscore the effectiveness of increasing treatment concentrations in controlling *C. pavonana* larvae and contribute insights into the physiological responses induced by the treatments, aligning with the insecticidal properties observed in previous studies, such as Idris (2015) on gambir leaf extract and *Plutella xylostella.*

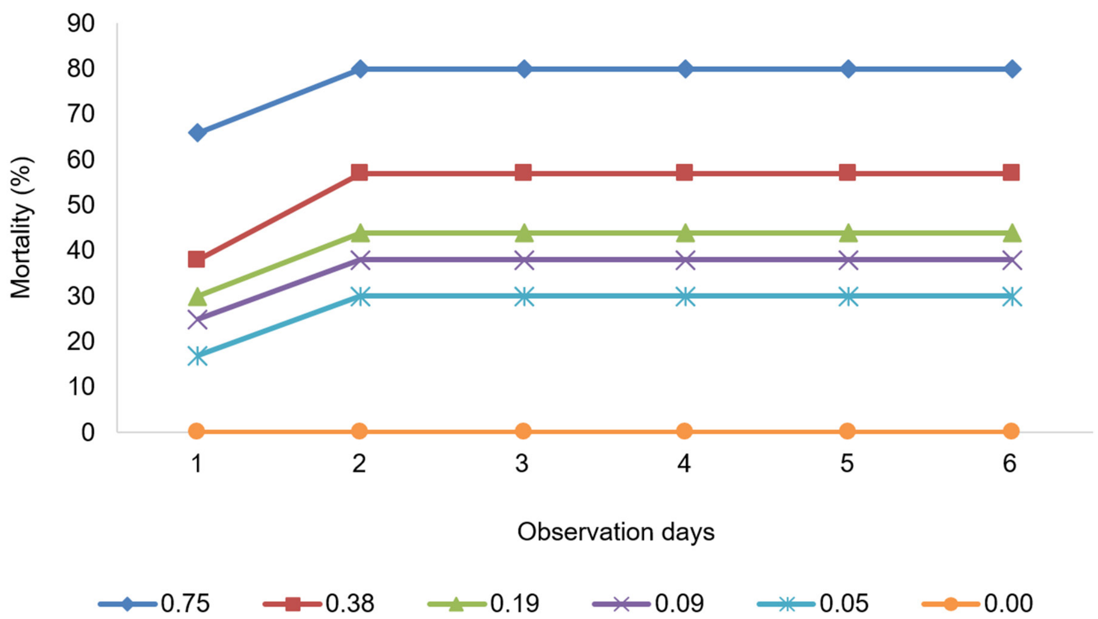

Fig. 1. The development of mortality of *Crocidolomia pavonana* larvae after being given solid gambir waste nanoemulsion during the follow-up test

Probit analysis of nanoemulsion solid gambir waste and liquid gambir waste, as shown (Table 3) for each larval mortality, has a regression slope value (1.12 ± 0.16 (solid waste) and 1.77 ± 0.22 (liquid waste) apart from that the LC_{50} and LC_{95} values of the nanoemulsion (solid waste) are smaller than the formulation (liquid waste) for *C. pavonana* larvae. This indicates that the difference between the two surfactant ingredients in the formulation did not significantly increase the mortality of *C. pavonana* larvae.

The results of the Particle Size Analyzer (PSA) analysis show that the nanoemulsion particle size for liquid gambir waste is 404.4 and solid gambir waste is 525.5, while for liquid waste, the Polydispersity Index was 0.52, and solid waste is 0.44. For Zeta Potential, liquid waste is -19.61, and solid waste is 25.54 (Table 4). The particle size of the nanoemulsion also contributes to larval death, so the smaller particle size makes it easier for the active ingredient to enter the leaf tissue. It causes the active ingredient to affect *C. pavonana* larvae after entering the larva's body through feeding and movement. PSA results show that both nanoemulsions have a particle size of <500 nm. Nanoemulsions are transparent or translucent emulsions with droplet sizes ranging from 20 to 500 nm (Kumar & Kumar, 2022). Nano size and surface properties of droplets significantly amplify the formulation's biological behavior (Sharma et al., 2020).

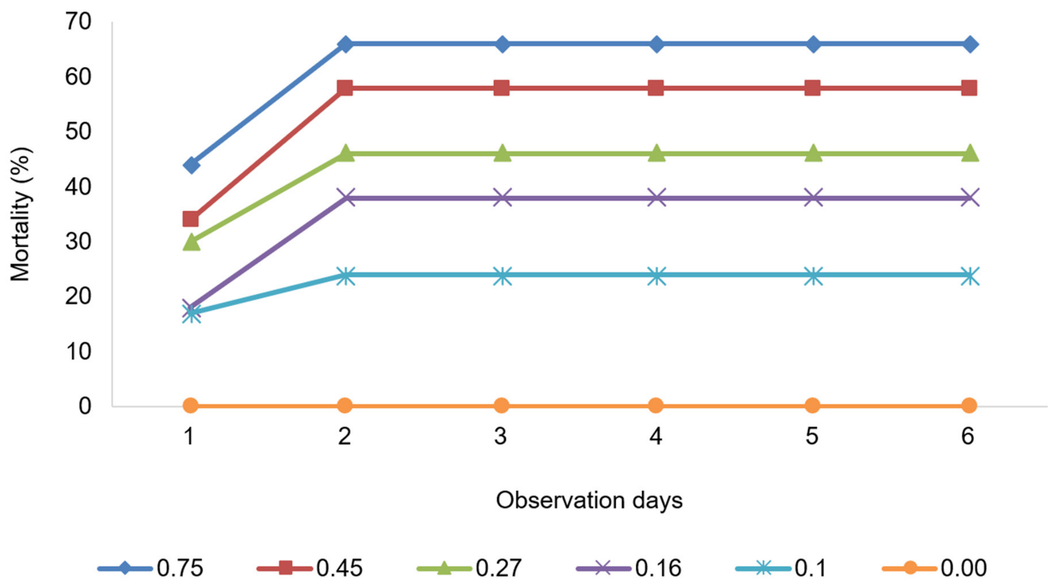

Fig. 2. The development of mortality of *Crocidolomia pavonana* larvae after being given liquid gambir waste nanoemulsion during the follow-up test

Table 3. LC_{50} and LC_{95} probit regression parameters of the relationship between the concentration of solid and liquid gambir waste nanoemulsion and citronella hydrosol on the mortality of *Crocidolomia pavonana* larvae

Value of b ± SE	LC_{50}(%)	LC_{95}(%)
1.12 ± 0.16 (Solid gambir waste)	0.22	2.44
1.77 ± 0.22 (Liquid gambir waste)	0.29	2.52

Table 4. Nanoemulsion analysis of solid gambir waste and liquid gambir waste using a Particle Size Analyzer (PSA) and Zeta Potential

Sample	***Particle Size*** **(nm) ± SD**	***Polydispersity Index*** **(PI) ± SD**	***Zeta Potensial*** **(mV) ± SD**
Liquid	80.88 ± 16.04	0.52 ± 0.05	-19.61 ± 4.65
Solid	105.10 ± 0.40	0.44 ± 0.03	-25.54 ± 4.30

CONCLUSION

The study suggests solid and liquid gambir waste can be eco-friendly alternatives to conventional insecticides. These waste forms exhibit the ability to impact the mortality of *C. pavonana*, with solid gambir waste demonstrating an LC_{50} concentration of 0.22 and liquid gambir waste at 0.29. Moreover, the LC_{95} values are 2.44 for solid gambir waste and 2.52 for liquid gambir waste.

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