



Full Length Article

Biological Control Test of Poultry Pest *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) with Mahogany and Papaya Seeds Extract

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Abstract

Currently, the control of poultry pest *Alphitobius diaperinus* is still a serious problem because the use of synthetic insecticides has been shown to cause health, resistance and environmental disturbances. Therefore, the exploration of environmentally friendly bioinsecticides is still relevant in order to reduce dependence on synthetic insecticides. This laboratory experimental study used a completely randomized design and was carried out in three stages (repellency test, mortality test and antifeedant test). Percentage of repellency (PR) and antifeedant activity were analyzed by ANOVA and continued with Tukey's test ($P < 0.05$). Mortality was analyzed descriptively and probit regression to predict of LC_{50} value. The results showed that PR value and insect mortality rates tended to increase in line with the concentration level of mahogany seed and papaya seed extracts. The highest PR value was shown in the C_{100} treatment in both extracts, 94.13 and 88.70%, respectively. The C_{100} treatment of mahogany seed and papaya seed extracts also recorded successive deaths of 100 and 96%, with LC_{50} values of 49.249 and 52.107%. The antifeedant effect was shown by lower feed consumption. The lowest feed shrinkage was found in the C_{40} treatment, at 63.56 and 52.66% respectively compared to the control group. The results of this study implied that mahogany and papaya seed extracts have the potential to be developed as bioinsecticides, especially in the control of *A. diaperinus*. © 2022 Friends Science Publishers

Keywords: *Alphitobius diaperinus*, Biological control, Mahogany seeds, Papaya seeds

Introduction

Today the use of synthetic insecticides in various sectors has become a serious threat to the environment, living organisms and food safety. The livestock sector, especially chicken farming, is inseparable from this problem. *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae), is one of the most common insect pests in commercial poultry farms. Economically these insects are detrimental to breeders because they cause serious repercussions. These insects easily adapt to artificial environments such as warehouses for storing agricultural products, as well as infesting stored grains and other processed products (Rumbos *et al.* 2020). *A. diaperinus* acts as a mechanical vector of several types of viruses, fungi and bacteria (Crippen *et al.* 2018; Soares *et al.* 2018) and accelerate the spread of pathogenic microorganisms in livestock.

Until now the chicken farmer still relies on synthetic insecticides, mainly from the pyrethroid and organophosphate groups. Nevertheless, the intensive and repeated use of insecticides became the cause of resistance

(Hawkins *et al.* 2019). Recent studies have shown evidence that *A. diaperinus* is beginning to be resistant to some synthetic insecticides of the pyrethroid and organophosphate (Velusamy and Ponnudurai 2019; Renault and Colinet 2021). Several types of insecticides such as alpha-cypermethrin, spinosad, and methyl pyrimiphos were no longer effective in controlling the population of *A. diaperinus* larvae (Zafeiriadis *et al.* 2021). Synthetic insecticides do have the advantage of being very efficient in killing insects in a relatively short period of time, but on the other hand, they can cause disruption of ecosystem function, poison various non-target organisms, and have a high tendency to accumulate in the environment. Many problems associated with the use of synthetic insecticides have prompted researchers to look for potential sources of natural insecticides that control insects. Exploration of bioinsecticide sources that have not been widely disclosed is waste from agricultural products, such as mahogany seeds (*Swietenia mahagony*) and papaya seeds (*Carica papaya*). This group of waste is familiar to the chicken farmer, it is abundantly available in various regions and its existence is often associated with the harvest season in a region in Indonesia.

Several previous studies have revealed that mahogany seeds and papaya seeds contain active compounds that are useful in the pharmaceutical and agricultural fields. The papaya seed extract is rich in oleic acid and triacylglycerols that have quite a lot of antioxidant activity (Samaram *et al.* 2015), antibacterial and antifungal (Sani *et al.* 2021). Papaya seed extract also has abated and toxic properties against the larvae of *Aedes aegypti* and *Aedes Albopictus* (Adayani and Subahar 2018; Abdullah *et al.* 2021). The papaya seed extract is proven to provide a toxic effect against cabbage plant pests *Plutella xylostella* and *Brevicoryne brassicae* (Ogbonna *et al.* 2021) as well as soybean plant pests *Spodoptera litura* (Bahuwa *et al.* 2022). A mahogany seed extract has toxic activity against caterpillars of *Spodoptera litura* and *Eudrilus eugeniae* (Dinesh-Kumar *et al.* 2018), larvae of *Aedes aegypti* (Vasantha-Srinivasan *et al.* 2021), larvae of *Tenebrio molitor* (Wida *et al.* 2020) and red mite *Tetranychus urticae* (Maldonado-Michel *et al.* 2022). This study aimed to analyze the repellency response, mortality rate and antifeedant activity of papaya seed extract and mahogany seed extract against *A. diaperinus* larvae. The evaluation was carried out by testing for contact toxicity for 72 h and the effect of repellent on adults of *A. diaperinus*, as well as antifeedant activity against the larvae of *A. diaperinus*. These three indicators can be used as a first step in developing bioinsecticides specifically for pest control of *A. diaperinus*.

Materials and Methods

The research was conducted at the Department of Biology, Faculty of Mathematics and Science, Universitas Negeri Semarang from February to May 2022.

Preparation of the extract

Papaya seeds and mahogany seeds were collected from the Temu Gesang herbal garden in Magelang Regency-Central Java, Indonesia. Seeds were dried in the shade, then blended and sifted until a fine powder was produced. Mahogany seed powder and papaya seeds were macerated for 3 x 24 h each using 95% ethanol solvent with the ratio of 5: 1. Furthermore, the material was filtered by using Whatman filter paper, and the obtained filtrate was evaporated using a rotary evaporator at a temperature of 40°C. The concentrated extract was stored in a refrigerator with a temperature of 5°C for further use. In this study, the concentrated extract was assumed to be an extract concentration of 100%. Qualitative phytochemical analysis was carried out for the identification of active compounds, terpenoids, flavonoids, alkaloids, phenols, phytosterols, and saponins by the standard method (Harborne 1993). GC-MS analysis was performed by using Clarus 500 Perkin-Elmer Gas Chromatography, while component identification was performed by matching the

MS spectrum of each peak with a standard mass fragmentation pattern from the Mass Spectral of the National Institute of Standards (NIST) Library.

Insects rearing

Adult *A. diaperinus* was collected from the broiler farm in Gunungpati Semarang and then reared in the Biology Laboratory of the Faculty of Mathematics and Science, Universitas Negeri Semarang. About 200 male and female adult ticks were kept in two insect containers. Commercial chicken feed was used in this breeding and pieces of cucumber fruit were added as a source of water while maintaining moisture. The maintenance room at the time of the study had a temperature of 28–29°C, with a humidity of 78–80%. In order to obtain relatively uniform larvae of *A. diaperinus*, in the first week of rearing, all adults were removed from the insect container to another maintenance container. The eggs left in the insect container were kept until larvae appear. It was these larvae that would be used for repellency tests, mortality tests and antifeedant activity.

Repellency test

The effect of repellent extract against adult *A. diaperinus* was observed using Y-Olfactometer glass tube (2.5 cm diameter). The tube has three interconnected passages. The length of each aisle is 15 cm, equipped with a lidded glass (10 cm long) that can be opened and closed. Aisle A was where to enter test animals, aisle B was for control and C was for placing treatment. The extracts were tested in 5 treatments of C₂₀, C₄₀, C₆₀, C₈₀, and C₁₀₀ extract concentrations, each of which was 100 µL and dripped into filter paper (1 x 1 cm). Filter paper that had been exposed to the extract was placed at the end of pipe C while the end of pipe B was given filter paper without extract (control treatment). Total of Thirty adults *A. diaperinus* was inserted through passage A and allowed to walk towards aisle B or C. Insects that showed the repellency response to the presence of the extract would eschew passage C and reverse course move to passage B. This preference test was carried out within a duration of 30 min and repeated five times. Data on the number of test insects located in passages B and C were used to calculate percentage repellency (PR) values, by the following formula (Ogbonna *et al.* 2021):

$$PR = \frac{NC - NT}{NC + NT} \times 100\%$$

PR= Percentage repellency; NC = number of insects entering the control treatment; NT= the number of insects that entered the treatment passage.

PR was classified descriptively into 6 categories as follows:

0.01% ≤ PR ≤ 0.1% (no repellent effect); 0.1% < PR ≤ 20% (very low repellent effect); 20% < PR ≤ 40% (low repellent effect); 40% < PR ≤ 60% (moderate repellent effect); 60% < PR ≤ 80% (high repellent effect); 80% < PR ≤ 100% (very high repellent effect).

Mortality test

Mortality tests were carried out in six levels of extract concentrations (C_0 , C_{20} , C_{40} , C_{60} , C_{80} , C_{100}) with an aqueous solvent as a diluent. Each treatment was repeated five times and each test used 20 larvae of *A. diaperinus*. Twenty of the larvae were put into a cup and then an extract of 100 μL was dripped directly to the groups of larvae and left for about a minute in each cup and then put two grams of feed. The entire experimental container was covered with gauze and maintained in a dark room. Larval mortality was observed at a time 24, 48 and 72 h after exposure to the extract. If the death percentage in the control group was found $< 5\%$, the data can be further analyzed, but if the percentage of deaths in the control group was in the range of 5–20%, then all the observational data were corrected first using the Abbott formula before doing statistical analysis. The 72 h mortality data were analyzed by using probit analysis to obtain the lethal concentration value (LC_{50}). The sublethal concentration (LC_{25}) value would be used as the maximum concentration limit in the antifeedant test.

Antifeedant test

The antifeedant effect in the study was observed on the basis of changes in the feeding ability of larvae, with indicators of a decrease in feed consumption. The test was carried out by measuring the ability of *A. diaperinus* larvae to consume feed after exposure to the extract in 5 concentration treatments (C_0 , C_{10} , C_{20} , C_{30} , and C_{40}). Each treatment was repeated 5 times and each repeat used 30 larvae. The larvae were first exposed to 100 μL of extract for 30 min in a plastic cup (diameter 5 cm, height 7 cm) a commercial chicken feed of 10 g was added to the plastic cup, and the cucumber fruit was split longitudinally as a source of water for the larvae. Maintenance was carried out in a dark room with a temperature of 28–29°C and humidity of 78–80%. Observations were carried out for 4 weeks. Each week, the number of feed depreciations and the number of larvae can be calculated so that the final data can be calculated in the form of average feed consumption (mg/larvae/week).

Statistical Analysis

The PR value and feed consumption were analyzed using variance analysis ANOVA and Tukey's test. The differences between the two extract sources were analyzed by using the student t-test, the percentage of mortality was analyzed descriptively and the value of LC_{50} was predicted by using probit regression.

Results

The qualitative phytochemical screening of mahogany and

papaya seed extracts previously carried out indicated the presence of alkaloids, flavonoids, saponins, phenols, triterpenoids, phytosterols, and tannins. Furthermore, the results of GC-MS analysis of mahogany and papaya seed extracts detected 34 and 31 types of active compounds, respectively, based on peak chromatograms, and as many as 10 compounds with a content of more than 1% were presented in Table 1 and 2. The results of analysis showed that both extracts contain active compounds from the fatty acid methyl ester (FAME) group such as oleic acid, methyl oleic and palmitic acid. Other FAME group compounds found were methyl linoleate, methyl stearate and methyl palmitic.

Repellency test

The repellent effect of the extracts on the larvae of *A. diaperinus* was analyzed by percentage repellency (PR) value and presented in Table 3. The results of the statistical analysis showed that the difference in extract concentration had significant ($\alpha < 0.05$) to the PR value, and after further analysis with Tukey's test, it was found that the PR at the concentration of C_{60} , C_{80} and C_{100} extracts was significantly different from the control treatment. Both extract sources showed the highest PR value found in the C_{100} treatment. However, statistically, mahogany seed and papaya seed extract did not significantly (Student's t-test; $P < 0.05$). C_{100} mahogany seed extract showed the highest PR value of 92.13% while papaya seed extract treatment reached 88.70%.

Mortality test

Mortality of *A. diaperinus* larvae after exposure to mahogany seed extract and papaya seeds was observed for 3 x 24 h. In 24 h observations, the highest percentage of deaths occurred in the C_{100} treatment in mahogany seed extract and papaya seeds, reaching 76 and 48% respectively (Fig. 1). However, in general, the mortality chart is increasing in line with the increasing concentration of the extract. At 72 h observations, 100% mortality only occurred in the C_{100} treatment of mahogany seed extract, while C_{100} papaya seed extract only reached 96% mortality.

The results of Probit's analysis of the mortality data of *A. diaperinus* larvae are known to have estimates of LC_{50} and LC_{25} as shown in Table 4. The lethal effect of mahogany seed extract that caused the death of test insects up to 50% was at a concentration of 49.249% while papaya seed extract was at 52.107%. The sublethal estimates of LC_{25} were at concentrations of 40.21 and 41.164%, respectively. With the prediction of this number, it can be interpreted that mahogany seed extract provides a greater toxic effect than papaya seed extract because the smaller the LC_{50} value, the higher the toxicity of the compounds contained in the extract. Furthermore, sublethal concentrations are used in antifeedant activity tests.

Table 1: List of chemical compounds of the *S. mahogany* seeds extracts based on GC-MS analysis

No	Ret. Time (min)	Chemical Compound	Chemical Formula	Mol. Weight	Rel. Area (%)
1.	34.64	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	6.64
2.	36.64	8,11-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294	1.80
3.	36.76	11-Octadecenoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296	1.91
4.	37.95	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	308	24.64
5.	38.06	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310	33.76
6.	38.15	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	1.13
7.	38.52	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	312	11.42
8.	40.01	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	1.02
9.	41.39	9,12,15-Octadecatrienoic acid, (Z, Z,Z)-	C ₁₈ H ₃₀ O ₂	278	1.66
10.	58.50	Fluprednisolone	C ₂₁ H ₂₇ FO ₅	378	3.95

Table 2: List of chemical compounds of the *C. papaya* seeds extracts based on GC-MS analysis

No	Ret. Time (minute)	Chemical Compound	Chemical Formula	Mol. Weight	Rel. Area (%)
1.	20.00	Benzene, (isothiocyanatomethyl)-	C ₈ H ₇ NS	149	4.68
2.	23.11	Urea, (phenylmethyl)-	C ₈ H ₁₀ N ₂ O	150	1.87
3.	28.68	Dibenzylamine	C ₁₄ H ₁₅ N	197	2.90
4.	28.75	2-Phenyl-2H-1,2,3-benzotriazole	C ₁₂ H ₉ N ₃	195	3.79
5.	34.70	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	5.71
6.	37.98	9,12-Octadecadienoic acid, ethyl ester	C ₂₀ H ₃₆ O ₂	308	2.63
7.	38.18	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	310	6.81
8.	38.62	cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	3.91
9.	38.77	6-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	7.81
10.	38.94	cis-Vaccenic acid	C ₁₈ H ₃₄ O ₂	282	5.31

Table 3: The PR value of the extract against *A. diaperinus*

Extract Concentration (%)	PR (%)	
	<i>S. mahogany seeds extract</i>	<i>C. papaya seeds extract</i>
C20	28.51 ± 15.048 ^a	25.25 ± 8.646 ^a
C40	28.96 ± 11.308 ^a	24.79 ± 13.005 ^a
C60	67.43 ± 18.560 ^b	77.94 ± 9.017 ^b
C80	86.79 ± 7.870 ^c	81.70 ± 5.265 ^{bc}
C100	94.13 ± 5.600 ^c	88.70 ± 5.252 ^c
Average	61.16 ± 30.685 ^a	53.07 ± 28.988 ^b

Notes: Different superscript letters in the same column indicate a significant difference ($P < 0.05$ by post hoc Tukey's test). Different superscript letters in the average line indicate a significant difference ($P < 0.05$ by Student's t-test)

Table 4: Estimates of LC₅₀ and LC₂₅ of mahogany seed and papaya seed extract

Estimation	Extract	
	<i>S. mahogany Seeds</i>	<i>C. papaya Seeds</i>
LC ₂₅	40.210	41.164
- Upper Bound	44.659	44.511
- Lower Bound	36.806	34.692
LC ₅₀	49.249	52.107
- Upper Bound	52.398	47.488
- Lower Bound	45.579	56.047

Table 5: Average feed consumption of *A. diaperinus* for 4 weeks

Extract Concentration (%)	Average of Feed Consumption (mg/larvae/week)	
	Papaya Seeds	Mahogany Seeds
C0	12.95 ^a	13.56 ^a
C10	11.53 ^a	9.67 ^b
C20	9.39 ^b	9.10 ^b
C30	9.16 ^b	6.15 ^b
C40	6.13 ^c	4.94 ^c
Average ^{ns}	9.83	8.68

Notes: Different superscript letters in the average column show a significant difference of 5% based on Tukey's test. The superscript of the letter 'ns' on the average line means that there is no significance

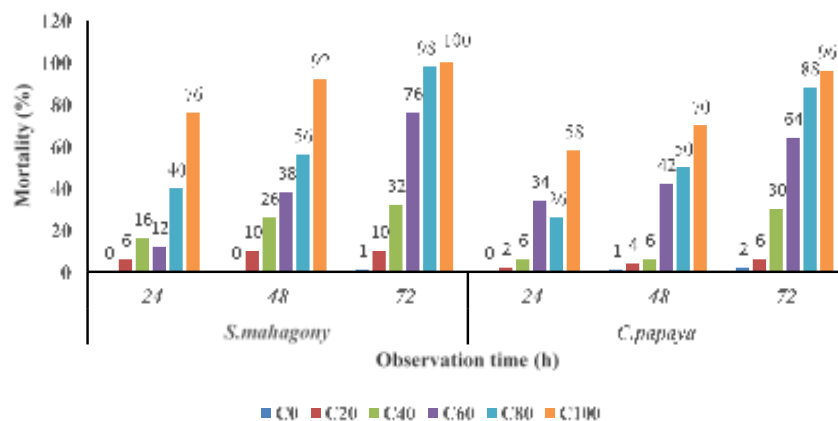


Fig. 1: Mortality of *A. diaperinus* larvae after exposure to mahogany seed and papaya seed extract was observed for 3 x 24 h

Antifeedant activity

The results of the variance analysis showed that mahogany and papaya seeds extract had a significant on reducing feed consumption of *A. diaperinus* larvae (Table 5). Feed consumption on the treatment tends to decrease in line with the decrease in extract concentration. The lowest feed consumption was found in the C₄₀ treatment in both extracts. The decrease in C₄₀ consumption of mahogany seed extract and papaya seeds reached 63.56 and 52.66% respectively compared to controls. This decrease indicates that mahogany seed extract and papaya seeds have an antifeedant effect.

Discussion

The results of the GC-MS analysis showed that both extracts contain active compounds from the fatty acid methyl ester (FAME) group such as oleic acid, methyl oleic and palmitic acid. Other FAME group compounds found were methyl linoleate, methyl stearate and methyl palmitic. Papaya seed extract mostly contains oleic acid and palmitic acid which are thought to act as important repellent compounds (Anggraeni and Laela 2020). The mahogany seed extract is detected to have oleic, linoleic, palmitic, and stearic acids, where these compounds are the main fatty acids that have the potential to produce insecticidal effects (Mohan *et al.* 2016; Mursiti *et al.* 2019). Benelli *et al.* (2016) and Wang *et al.* (2014), mentioned that extracts containing a wide variety of active compounds are more effective at influencing insect behavior compared to extracts containing only a single compound. However, whether the effect is strong or not depends on the dose administered.

The results of the statistical analysis of the repellent activity in *A. diaperinus* larvae exposed to mahogany seed and papaya seeds extract showed that the difference in extract concentration had a real effect ($\alpha < 0.05$) on the PR value, and after further analysis with the Tukey's test, it was found that the PR at the concentration of C₆₀, C₈₀, and C₁₀₀ extracts was significantly different from the control treatment. Both extract sources showed the highest PR value

found in the C₁₀₀ treatment. However, statistically, mahogany seed and papaya seed extract did not significantly (Student's t-test; $P < 0.05$). C₁₀₀ mahogany seed extract showed the highest PR value of 92.13% while papaya seed extract treatment reached 88.70%. A high PR value indicates a stronger insect repellency response (Chakira *et al.* 2017).

The content of active compounds of the essential oil group and the main fatty acids in both extracts is thought to affect the response of insects. In addition to causing specific odors, the extract is thought to affect the performance of neurotransmitters of the acetylcholinergic and octopaminergic systems. Both are neurotransmitters that modulate the level of locomotor activity in insects (Jankowska *et al.* 2017). If *A. diaperinus* gets an excitatory odor from the extract, the neurotransmitter acetylcholine will initiate to stimulate the muscles to contract resulting in movement in the body of *A. diaperinus* to stay away from the source of the odor.

In 24 h observations, the highest percentage of deaths occurred in the C₁₀₀ treatment in mahogany seed extract and papaya seeds, reaching 76 and 48% respectively (Fig. 1). However, in general, the mortality chart is increasing in line with the increasing concentration of the extract. At 72 h observations, 100% mortality only occurred in the C₁₀₀ treatment of mahogany seed extract, while C₁₀₀ papaya seed extract only reached 96% mortality. This situation indicates that the extracts of mahogany seeds and papaya seeds have toxic properties to the larvae of *A. diaperinus*, but the effect is slow. Previous research stated that papaya seed extract can be a contact poison in the larvae of *Plutella xylostella*, *Brevicoryne brassicae*, and mosquitoes (Bongalon *et al.* 2019). Similarly, mahogany seed extract is known to contain secondary metabolites that have antifeedant properties, contact toxins and growth reduction inhibitors (Arazo *et al.* 2017).

The toxic effect works slowly because insects have their own mechanisms for dealing with toxic substances. Insects can express metabolic adaptations that result in modifications of plant chemicals that are ingested or exposed

to make them less toxic, easier to transport or excrete, or functionally altered to the benefit of the insect itself (Dobler *et al.* 2012). Thus, the larvae of *A. diaperinus* that remain viable after a 72 h treatment are likely to have a good defense system so that they can overcome exposure to toxic substances from mahogany seed extract and papaya seeds. Conversely, the content of secondary metabolite compounds in high concentrations can cause insects to fail in the metabolism of toxic substances. The extract, which is presented by direct contact with the insect's body, will work as a contact poison by damaging the external physique (cuticle), causing insects to lose body fluids slowly and within a certain period of time can cause death (Bahuwa *et al.* 2022).

The average feed consumption on the treatment tends to decrease in line with the decrease in extract concentration (Table 4). The lowest feed consumption was found in the C40 treatment in both extracts. The decrease in C₄₀ consumption of mahogany seed extract and papaya seeds reached 63.56 and 52.66% respectively compared to controls. This decrease indicates that mahogany seed extract and papaya seeds have an antifeedant effect. In general, the mechanism of antifeedants is to inhibit the response of receptor cells that are sensitive to eating stimulants such as cravings for eating or the sense of taste. Compounds that are antifeedants can inhibit insect eating through sensory perception, such as having an unpleasant taste in insects (Chapman 1995). The presence of aromatic compounds also makes insects lose their appetite for food, thereby reducing or stopping eating. Some essential oils and monoterpenes are reported to have inhibitory effects on the enzyme amylase and other digestive enzymes that participate in metamorphosis and physiological function (Arasu *et al.* 2013). Khan (2021) who tested mahogany seed oil to control warehouse insects of *Rhyzopertha dominica* concluded that the compound was shown to have toxic properties and cause weight shrinkage. In addition to essential oils, alkaloids also provide antifeedant effects against insects and have the potential to be bioinsecticides for insect pest control (Manosalva *et al.* 2019). The other finding reported the presence of synergistic effects of several phytochemical compounds contained in plant extracts, which play an important role in their natural defense against insect attacks (Marin *et al.* 2020). Phytochemical compounds, either separately or synergistically are capable of providing antifeedant effects, toxic effects, or acting as precursors to the defense system of plants. *Swietenia macrophylla* King seeds contain limonoids that can be used as bioinsecticides because they have a repellent, antifeedant, and toxic effect against insects. Limonoids are derivatives of Azadirachtin that can affect appetite (Telrandhe *et al.* 2022). Azadirachtin affects hormonal metabolism in the brain of insects and indirectly modifies the synthesis of juvenile and pheromone hormones (Hummel *et al.* 2012; Fathoni *et al.* 2013). In insects, 20-hydroxyecdysone and juvenile hormones are the main

hormones that regulate the regulation of growth, and metamorphosis.

Conclusion

The results of this study proved that the extracts of mahogany seeds and papaya seeds contain active compounds that cause mortality effects, repellent effects, and antifeedant effects on *A. diaperinus* insects. This potential can be used as the basis for the development of bioinsecticides based on mahogany seeds and papaya seeds, especially for the control of *A. diaperinus*.

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Author Contributions

PW developed the concepts and also designed the experiments, NSub Collection, prepare extract and phytochemicals screening using standard procedures GC-MS method, NSet carried out experiments, data recapitulation, and statistical analysis, LA responsibility on the extract and insect preparation, ARN and RMKR carried out experiments and record data.

Conflicts of Interest

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

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics Approval

This research material doesn't require Ethics Approval

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