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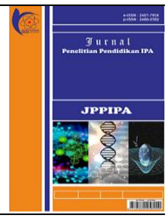

Potential of Binahong Leaves (*Anredera cordifolia* Ten.) and Shallot Skin (*Allium cepa* L.) Extract as Biopesticides for Biological Control of Rice Bug (*Leptocorisa oratorius* F.)

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Abstract: The rice bug is a pest causing decrease rice production. Meanwhile, binahong leaves and shallot skin contain potential materials for making biopesticides. This research aimed to analyze potential of binahong leaves and shallot skin extract as biopesticides and toxicity tested against rice bug. The research was carried out using Completely Randomized Design, biopesticides were made in various concentrations, 0% as a control; 10; 20; 30; 40; and 50. The extract was calculated for yield, pH, and phytochemical screening. Then, biopesticide sprayed on the pests at 24, 48, 72, 96, 120, 144, and 168 hours to observe mortality, LC_{50}, morphology, behavior, biotic, and data analysis using ANOVA followed 0.05 Tukey test. The results showed the extract has potential as a biopesticide with yield value 8,3 – 12; pH value 4,84 – 5,37; and positive secondary metabolite. Meanwhile, toxicity tests showed the highest mortality at concentration 50%, LC_{50} at 144th hours and 168th hours was 4,373 and 2,002. Besides that, biopesticides changes morphology and behavior pest. Observations abiotic factors showed that rice bug can survive in temperature range of 22,75 – 25°C and relative humidity of 77 - 84%. In the future, it hoped biopesticides can be commercialized to reduce dependence on chemical pesticides.

Keywords: Biopesticides; Phytochemical screening test; Rice Bug; Toxicity test

Introduction

Rice is an agricultural food crop commodity in Indonesia that has an important role in people's lives with approximately 90% consumption as the staple food of Indonesian (Donggulo et al., 2017). According to the Food and Agriculture Organization (FAO) United Nations, the agricultural sector will very serious challenges in the next 35 years as a result of the global population increasing up to 30% in 2050 with global population growth which will reach 9.3 billion. At the same time, the need for rice food will increase from 8.4 billion tonnes to 13.5 billion tonnes (Saragih & Panggulu, 2021). One of the problems with rice productivity is caused by pest attacks (Gama et al., 2021).

The rice bug (*Leptocorisa oratorius* F.) is a pest belonging to the Hemiptera Order, Alydidae Family. This pest will suck the rice grains and attack the rice plants during the milk ripening phase, causing the rice grains to become empty (Wati et al., 2021). Rice bugs are one of the pests that can cause a decrease in the quality of grain and rice yields can losses of up to 50%, even heavy attacks of rice bugs can cause the rice yields decrease up to 100%, research also shows that 100.000 rice bugs per hectare can cause a decrease up to 25% rice yield (Salim & Suryati, 2021).

Meanwhile, the management of rice bug pests currently still uses chemical pesticides which can cause environmental pollution problems, pest explosions, pest resistance, and resurgence, killing non-target organisms and natural enemies of plant pests (Asikin & Lestari,

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2020). Chemical pesticides are not easily and quickly degraded, chemicals will accumulate at trophic level of food chain in the ecosystem which can cause an increase chronic lethal or sub-lethal toxicity (Suripto, Ahyadi, et al., 2023). The concept of biological control is a wise concept that can be used to handle pests (Saenong, 2017). Biopesticides are natural ingredients to control pests that are environmentally friendly and safe for human health (Nik et al., 2023). Biopesticides are easily decomposed because do not have residues, do not cause side effects on the environment, the materials are easy to obtain and easy for farmers to adopt (Mustapa et al., 2023). Various plant species can be identified as potential biopesticides for pest control (Febri et al., 2024). Plants contain secondary metabolites that have the potential to be used as raw materials for making biopesticides (Saenong, 2017). Secondary metabolites can cause pest death as toxins through various mechanisms such as respiratory toxins, stomach toxins, and neurotoxins (Embrikawentar & Ratnasari, 2019).

However, biopesticides developed currently are not completely safe for their users. Research study conducted by Sianipar et al. (2020) binahong leaves extract with the addition of Tween 80 can be used to suppress the population of brown plant hopper (*Nilaparvata lugens*) in rice plants. But, the additional use of Polysorbate 80 (Tween 80) in biopesticides has health effects for users (Sujarwati & Nurcandra, 2023). Polysorbate 80 (Tween 80) is a synthetic surfactant used as a solvent, stabilizer, and emulsifier in various fields (Schwartzberg & Rudolph, 2018). Tween 80 (Polysorbate 80) irritates and is dangerous if skin contact occurs, irritation if direct contact with the eyes, dangerous if swallowed and inhaled because it contains irritants which are substances that trigger inflammation. The use of biopesticides with Polysorbate can have an impact on farmers health with the largest percentage of skin irritation being 32.95% (Sujarwati & Nurcandra, 2023).

One way to overcome this problem is offering the use biopesticides from binahong leaves extract and shallot skin as an environmentally friendly biological control without the use of Polysorbate/Tween 80. Binahong (*Anredera cordifolia* Ten.) is a plant belonging to the Caryphyllales Order, Basellaceae Family. Binahong leaves have 28.14 mg/g saponin content (Rohaeni et al., 2024). Whereas, saponin content contained in plants can be used to replace synthetic surfactants, plant saponins have the highest surfactant properties of all bioactive compounds. Saponins can be used as solubilizers and stabilizers in various fields (Rai et al., 2021). Meanwhile, shallot (*Allium cepa* L.) is one of the plants belonging to the Liliales Order, Liliaceae Family (Nanda et al., 2022). Shallot skin are waste produce by most home industries and cannot be fully utilized (Rahayu et al., 2015). HPLC-DAD analysis showed that shallot skin extract has the highest content of the main compound cyanidin 3-O-(6″-malonyl-laminaribioside) (Stoica et al., 2021). Cyanidin is a part of anthocyanin which is part of the flavonoid group, which mostly brightens the outer layer of the onion skin, around 60% (Supriatno et al., 2023).

This research has a novelty in finding the composition of biopesticide from binahong leaves and shallot skins that have never been found before as biopesticides without the addition of Polysorbate/Tween 80. Therefore, this study aims to analyze the potential of a mixture binahong leaves extract and shallot skin extract as a biopesticide and toxicity test of biopesticide against the rice bugs pest.

Method

This research was conducted through several stages presented through a flowchart (Figure 1).

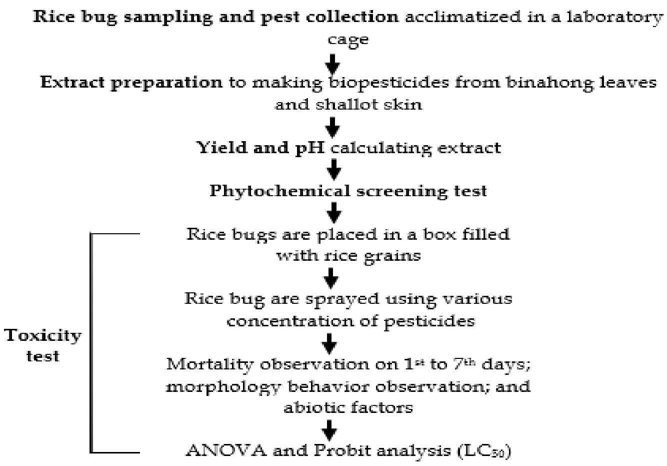


Figure 1. Research workflow

Rice Bugs (Leptocorisa oratorius F.) Preparation

A sampling of rice bug pests was carried out in a farming area in Ketindan Village, Lawang, Malang, East Java (Latitude: 7°50'10.39"S, Longitude: 112°41'10.54"E). The sampling area is 1000 m² (Figure 2). The rice bug pest was obtained using the sweeping net technique by swinging the net zig-zag in the plot of plant area and the pests that had been caught were then put in a small container and then the pests were taken to the laboratory (Pasaru & Wahid, 2013). The rice bug pest used for research is the 5 nymph phase because in this phase there is a rapid molting period and a critical period for the rice bug (Embrikawentar & Ratnasari, 2019). The obtained pests were then taken to the Plant Protection Laboratory, Balai Besar Pelatihan Pertanian (BBPP) Ketindan Malang. The pests were then acclimatized in a laboratory cage and fed rice grains for three days (Gama et al., 2021).

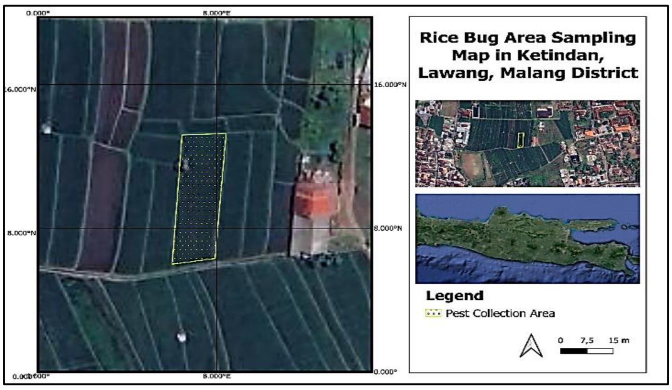


Figure 2. Sampling location of rice bug

Extracts Preparation

Biopesticides are made using the extraction method, 3000 g of binahong leaves were weighed, washed, and air-dried (Sianipar et al., 2020). 1000 g of shallots skin were weighed, washed, and dried in the open air (Mardiah et al., 2017). Each ingredient is ground without adding water using a blender (Sianipar et al., 2020). Then maceration was carried out using 96% ethanol with a ratio of 1:4 w/v for three days (Dewitasari et al., 2017), and continued with maceration for 3 days at room temperature and evaporated using a rotary evaporator (Sianipar et al., 2020) The results of the maceration were evaporated at the Pesticide Toxicology Laboratory, Department of Plant Pests and Diseases, Brawijaya University. Evaporation is carried out with a vacuum rotary evaporator (Heidolph Hei-VAP Rotary Evaporators) at a temperature of 40^{o}C, and a vacuum pressure of 175 bar (Assagaf et al., 2012).

Yield and pH Calculating

The extract resulting then calculates % yield according to Abbas et al. (2021) using the following Formula 1.

$$\text{Yield (\%)} = \frac{\text{Weights (Solvent free extract) x 100}}{\text{Weight (Dried extract)}} \quad (1)$$

The pH of the extract is calculated using litmus paper and a pH meter, measured pH using litmus paper by dipping the litmus paper into the extract, then waiting for the color change on the paper and comparing it with the standard color table. Meanwhile, measured using a pH meter by dipping the pH meter electrode into the extract then the measurement result will automatically be displayed on the pH meter (Checchetti & Lanzo, 2015).

Phytochemical Screening Test

Phytochemical screening after 3 months storage period was conducted to determine secondary metabolites of the extract. The phenol content test was carried out by adding 2% $FeCl_3$; 1% HCl and Mg powder were added to the flavonoid test; 1% $FeCl_3$ was added to the tannin content test; hot water was added to the saponin content test; chloroform, sulfuric acid, and acetic anhydrous were added to the triterpenoid and steroid content test (Souhoka et al., 2021).

Biopesticide Toxicity Test

Toxicity tests were carried out using a Completely Randomized Design (CRD), biopesticides were made in various concentrations, 0% as a control; 10%; 20%; 30%; 40%; and 50% binahong leaves extract, and 5% shallot skin extract were added for each concentration. There were 6 treatments combination and 5 replications, 300 rice bugs is used in this study.

The rice bug pests were placed in each box containing ten rice bug pests and then a biopesticide was applied (Karsidi et al., 2014). Observations were made at the 24, 48, 72, 96, 120, 144, and 168 hours. Rice bugs were given cooked milk grains as food (Gama et al., 2021). The spraying application of biopesticide extract was carried out at 4 pm (Karsidi et al., 2014). Pesticide application is carried out using the spray method. Spraying of biopesticides in each treatment was carried out by spraying 10 ml with a hand sprayer (Embrikawentar & Ratnasari, 2019). The effect of the toxicity of biopesticides on rice bugs was observed in mortality, Lethal Concentration (LC_{50}), morphology pest, behavior pest before and after applied biopesticide, and abiotic factors (temperature and humidity).

Data Analysis

Toxicity test data analysis was carried out using SPSS 16.0 software with a significance value of $p<0.05$ ANOVA followed by the Tukey test to determine the significance of pest mortality. Data analysis on changes in behavior and morphology of pests was carried out descriptively by observing during observation activities (Karsidi et al., 2014). Lethal Concentration (LC_{50}) value was determined using probit analysis of each mortality data to determine their pesticide selectivity (Suripto et al., 2023).

Result and Discussion

Potential of Binahong Leaves Extract and Shallot Skin Extract as Biopesticides

Based on the yield value, composition of the biopesticides, binahong leaves extract has a yield percentage of 12% and shallot skin extract of 8.33%. The weight of the final extract (evaporated extract) obtained and the initial simplicial can determine the percentage yield value. The initial weight of binahong leaves simplicia is higher because it is used as the main raw material for making biopesticides (Table 1). This is also in accordance with the other research, that have shown the yield value of binahong leaves extract varies between 1.64% to 13.98% (Susanty & Yudhistirani, 2018). Meanwhile, the yield value of shallot skin extract varies between 5.83% to 13.39% (Badriyah et al., 2022). A high yield value indicates that the extraction of bioactive compounds in plants has been effective. The higher yield value in the extract causes a higher content of substances that can be extracted from raw material (Senduk et al., 2020). The higher bioactive compounds of secondary metabolites in biopesticides, cause the higher toxicity of the biopesticide, so the toxicity against the pests will also be higher (Limbong et al., 2021).

Table 1. Yield Percentage of Binahong Leaves and Shallot Skin

Aspect	Binahong leaves	Shallot skin
Evaporated extract (gram)	250	120
Initial simplicial (gram)	3000	1000
Yield (%)	8.33	12.00

Meanwhile, calculating the pH value using a pH meter and litmus paper shows that both of extracts have a pH value of 4-5, pH value indicates that the biopesticides are acid (Table 2). One of the factors causing the mortality of the pest is acidic pH (Gama et al., 2021). The acidic pH value can influence the toxicity of pesticides on pest deaths. The acidic pH value indicates the number of biopesticide ingredients that decompose to form acidic chemical compounds Isa et al. (2019) pH can affect enzymes related to insect metabolism, acidic pH can cause the activity of enzymes for metabolism to be inhibited, causing disruption to the insects biological functions and can cause animal mortality (Delfita, 2014). The poison in biopesticides has a mechanism that can cause pH of the animal blood to increase, which can cause blood clots and stop blood circulation (Herlinda, 2016). The high toxicity of biopesticides can cause paralysis of the intestinal walls, then the intestinal pH will decrease, increase in blood pH, and can cause pest death (Salaki & Jackson, 2022).

A phytochemical screening test of biopesticides was carried out after 3 months storage period in the laboratory. The results phytochemical screening test of biopesticides showed that after 3 months storage period, the biopesticides were positive for containing secondary metabolite compounds including phenols, saponins, tannins, flavonoids, triterpenoids, and steroids. However, binahong leaves extract was negative for triterpenoids and shallot skin was negative for steroids (Table 3). Binahong leaves extract and shallot skin had positive phenol test results as indicated by the solution changing to black (Figure 3a). Positive test results for flavonoids are indicated by changes the solution to red and orange (Figure 3b). Positive test results for tannin are indicated by changes the solution to black (Figure 3c). Positive test results for saponins are indicated by the formation of froth or foam as high as ±3 cm (Figure 3d). Binahong leaves extract was negative for triterpenoids but positive for steroids, while shallot skin extract was positive for triterpenoids and negative for steroids. Changing the color of the sample to red indicates that the sample is positive for containing terpenoids while changing the color to green indicates that the sample is positive for containing steroids (Figure 3e).

Table 2. The pH Value of Binahong Leaves Extract and Onion Skin After Evaporation

Test	Binahong leaves	Shallot skin
pH meter	5.25 – 5.37	4.84 – 4.99
Litmus paper	5	4

Screening test result is a little different from the other research, that have shown binahong leaves extract was positive triterpenoids (Basyuni et al., 2017). Whereas, shallot skin was positive for steroids (Anggarani et al., 2021). Negative secondary metabolite can be due to storage environmental conditions. Environmental conditions of storage such as temperature, humidity, and light intensity can ffect the secondary metabolite content in the extract. In general, the ideal conditions for storing extracts are at relatively low temperatures because high temperatures can destroy the molecular structure of secondary metabolites, causing secondary metabolites to not be detected in the extract. Stable humidity can maintain the stability of biopesticides and light-intensity storage in room conditions that are protected from direct sunlight, and can help inhibit biopesticide degradation due to ultraviolet light (Mulyani et al., 2022; Poncowati et al., 2022). Besides that, longer daylight will promote the production of metabolite secondary (Astuti et al., 2023).

Table 3. Phytochemical Screening Test Results of Binahong Leaf Extract and Shallot Skin

Secondary metabolites	Test method	Binahong leaves	Shallot skin
Phenol	Ethanol + $FeCl_3$ 2%	Positive (+)	Positive (+)
Saponins	Hot water (foam)	Positive (+)	Positive (+)
Tannin	Distilled water + $FeCl_3$ 1%	Positive (+)	Positive (+)
Flavonoids	Mg + HCl	Positive (+)	Positive (+)
Triterpenoids	Chloroform, sulfuric acid, and acetic anhydrous	Negative (-)	Positive (+)
Steroids	Chloroform, sulfuric acid, and acetic anhydrous	Positive (+)	Negative (-)

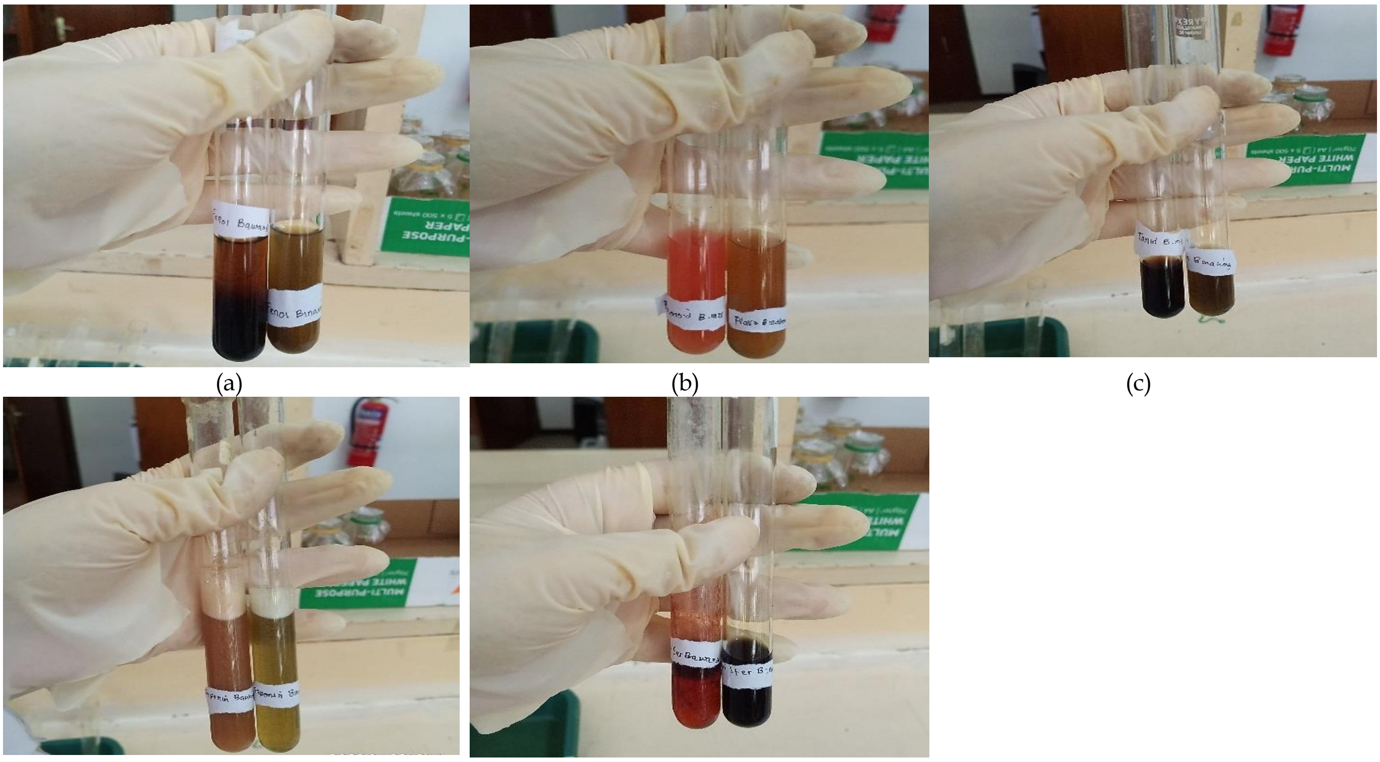


Figure 3. Screening test results, (a) Phenol test; (b) Flavonoid test; (c) Tannin test; (d) Saponin test; (e) Test for steroids and triterpenoids

Toxicity Test on Mortality of Rice Bug Pests

The highest percentage of mortality was at concentrations of 40% and 50% with a mortality of 100%, while the lowest mortality was 0% (control) with a mortality of 0% and a concentration of 10% with a mortality of 8%. Treatment at concentrations of 40% and 50% at the 144th hours and 148th is an effective concentration because it can provide 100% mortality of the rice bug pest. Biopesticide treatment gave high mortality along with the high concentration given (Table 4). This result is also in accordance with the other research, that have shown increasing the concentration of extract can cause increased animal mortality, low concentrations have low toxic levels so that animals mortality is low, while high concentrations have high toxic levels so that animals mortality is also high (Supriatno et al., 2024). The percentage of mortality of rice bug pest is also influenced by concentration, the higher concentration given to the treatment cause the higher the mortality of the rice bug. The higher the concentration causes the higher compound content in biopesticides (Karsidi et al., 2014). The percentage of mortality of rice bugs from each concentration was tested using the 5% Tukey test, the differences are shown by the letters on each bar. The Tukey HSD test (Significance 5%) shows different mortality percentages at each treatment concentration (Figure 4).

Table 4. Average Percentage of Rice Bug Mortality Per Day

Concentration (%)	Mortality (%)							
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours	148 hours	Mean ± Std. Dev
0%	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a	0.00 ± 0.00
10%	8.00a	44.00b	54.00b	66.00bc	78.00cd	82.00cd	92.00d	60.57 ± 28.47
20%	8.00a	52.00b	70.00c	82.00cd	88.00cd	92.00d	96.00d	69.71 ± 31.08
30%	14.00a	62.00b	78.00c	84.00cd	88.00cd	96.00d	98.00d	74.29 ± 29.20
40%	18.00a	64.00b	80.00c	84.00cd	90.00cd	100.00d	100.00d	76.57 ± 28.68
50%	22.00a	66.00b	82.00c	88.00cd	96.00d	100.00d	100.00d	79.14 ± 27.93

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% significant level (α) according to Tukey Test

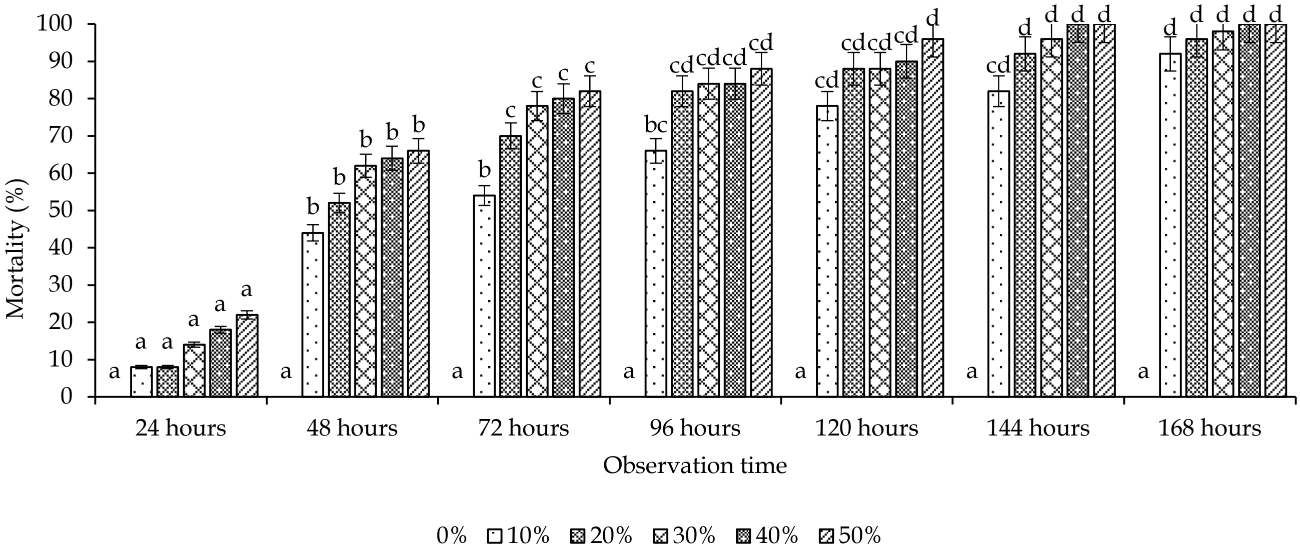


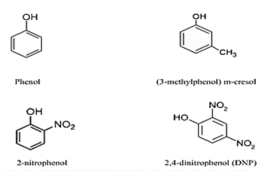
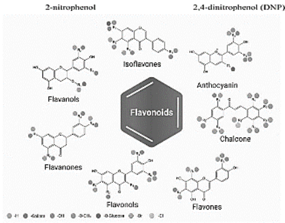
Figure 4. Percentage of rice bug mortality based on observation time (day), using SPSS Tukey Test at the 5% significant level

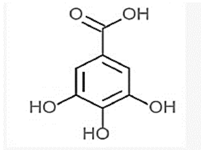
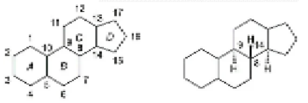
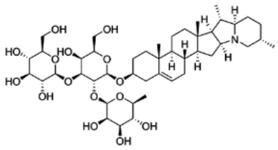
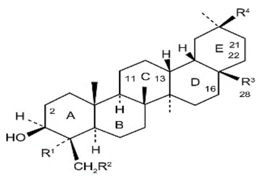
The toxicity of biopesticides that cause mortality in rice bugs is due to the secondary metabolites contain in biopesticides which have roles as respiratory poisons, stomach poisons, contact poisons, and neurotoxins (Table 5).

The mechanism of biopesticides as neurotoxins occurs due to the inhibition of cholinesterase enzyme that regulate nerve performance and transmit stimulation to nerve receptors in muscle and gland cells. Inhibited cholinesterase enzymes will cause the active insects movements, and then cause the nervous system uncoordinated and muscle spasms and cause the death of insects (Embrikawentar & Ratnasari, 2019).

The mechanism of biopesticides as stomach poisons can influence insect metabolism after eating, poisons that enter the insect body will circulate in the blood and poisons in the blood can cause the death of insects (Fauzana & Nurul, 2018). When biopesticides enter the insect body through food, these toxins will inhibit cell metabolism by inhibiting electron transport in mitochondria, and then energy formation does not occur in the cells and the cells cannot carry out activities which can cause the death of insects (Sa'diyah et al., 2013). The poison that enters the insects body can cause the surface tension of the mucous membrane to decrease and the walls of the digestive tract to become corrosive, the activity of digestive enzymes decreases and the insects appetite decreases (Syah & Kristanti, 2016). Toxic substances in biopesticides will spread to all parts of the body's cells through the circulatory system and will cause the body's circulatory system to be disrupted. Disrupted enzyme secretion will cause the digestive process to be disrupted and animals lack energy which can result in mortality (Supriatno et al., 2023)

Table 5. The Role of Secondary Metabolites in Biopesticides

Secondary metabolite	Structure	Activity	Reference
Phenol		Contact poison	(Surkatti & Al-Zuhair, 2018)
Flavonoids		Respiratory poison	(Drețcanu et al., 2022; Embrikawentar & Ratnasari, 2019)

Secondary metabolite	Structure	Activity	Reference
Tannin		Stomach poison	(Bar & Ganguly, 2022; Embrikawentar & Ratnasari, 2019)
Saponins		Stomach poison	(Embrikawentar & Ratnasari, 2019; Mohaddes et al., 2019)
Steroids		Stomach poison and contact poison	(Gomes et al., 2023)
Triterpenoids		Nerve poison	(Embrikawentar & Ratnasari, 2019; Weng et al., 2011)

The mechanism of biopesticides as contact poisons occurs through direct contact of the biopesticide with the insects body. The toxins can enter the insects body through the skin, mouth, or trachea. Secondary metabolite compounds as contact poisons will enter through the epicuticle into the tissue under the insects integument (cuticle), sensory glands (trachea), and other organs that are connected to the cuticle. Secondary metabolite compounds in biopesticides as poisons can dissolve the fat or wax layer on the cuticle so that these compounds can penetrate the insects body (Permatasari et al., 2021).

The mechanism of biopesticide as a respiratory poison occurs due to disturbances in the working system of respiratory tract in the rice bug so that it can cause insects death, the toxins will enter through the stigma and spiracles in the respiratory tract and then reach the trachea and causing problems the respiratory system (Embrikawentar & Ratnasari, 2019).

Meanwhile, the concentration needed to kill 50% of pests based on SPSS 16.0 Lethal Concentration (LC_{50}) analysis was obtained starting from 144th hours and 168th hours. The LC_{50} result at the 144th hours was 4,373 and at the 168th hours was 2,002. The longer the application time the LC_{50} value will be lower due to the accumulation of toxin compounds (Table 6). The toxicity of biopesticides that can cause insects death can be measured through LC (Lethal Concentration). The LC_{50} value gets lower as time increases due to the accumulation of toxins in the insect body. Accumulation of biopesticide toxins in the insect body can occur when toxins accumulate excessively in the insect body (Arfiati et al., 2019).

Table 6. The value of LC_{50} day 6 and day 7

LC_{50} (%)	6 (144 hours)	7 (168 hours)
	4.37	2.00

Pest Morphology and Behavior

The morphology of the rice bug before spraying with biopesticides had a light brown body, long antennae standing upright, segments of stomach with each segments having a black spot, while the morphology of the rice bugs after spraying had a blackish body, the antennae were drooping not standing up straight, segments on the body becomes invisible and the head is blackish (Figure 5). Apart from that, biopesticide treatment also provided a change in behavior, before spraying the rice bug pest was active and moved quickly and more often moved on the top surface of the box treatment. However, after spraying with biopesticides the rice bug was immediately avoided and moved to the bottom surface of the box treatment. Then the rice bug was silent, limp, and didn't move for several minutes.

The morphology of the rice bugs and the giving of the extract are related when the rice bugs die, the body makes morphological changes (Ningsih et al., 2016). This result is also in accordance with the other research, that have shown the change in body color to black is caused a response as a defense of the rice bug body against or a result of the activity phenoloxidase enzyme (Sidauruk & Hafiz, 2021). The phenoloxidase enzyme is an enzyme that is involved in melanin formation, found in arthropod skin and responsible for the melanization process of arthropod skin. Then this enzyme will catalyze the hydroxylation of monophenols and the oxidation of phenols into quinones for the melanization process as a response to the presence of foreign object (Manoppo & Kolopita, 2014).

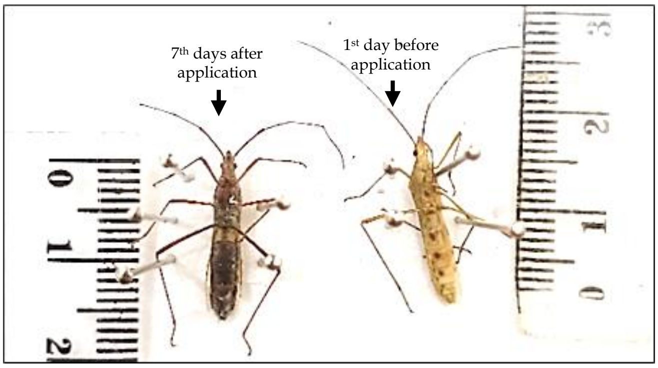


Figure 5. Morphology of rice bug on 1st day before application and 7th days after application biopesticide

Behavior changes in the rice bug are caused by secondary metabolites contained in biopesticides (Embrikawentar & Ratnasari, 2019). Rice bug that were sprayed before become silent, weak, and didn't move because of the obstruction of the work of cholinesterase enzyme, which regulates nerve function and transmits stimulation to nerve receptors in muscle cells and glands. The cholinesterase enzyme encounters work obstacles, which will cause changes in the movement of the insect, causing the nervous system to work uncoordinated and will cause muscle spasms that can ulimately lead to the death of the insect. The pests slow movements and flying activity will decrease. The rice bugs will land on the rice then move their wings, front legs, and rub against their antennae and decreasing feeding activity (Karsidi et al., 2014). The rice bug pest used for research is the five nymph of the rice bug and in this phase the molting period which occurs quickly and is a critical period so that when the extract is applied it can have a contact with poison effect and cause death to the pest and the partition between the segments of the rice bug. The rice bugs at the molting stage have a thinner cuticle than other parts (Embrikawentar & Ratnasari, 2019).

Table 7. Temperature and Relative Humidity

Time Observation	Temperature (°C)			Relative Humidity (%)		
	7:00 AM (morning)	12:00 PM (noon)	4:00 PM (afternoon)	7:00 AM (morning)	12:00 PM (noon)	4:00 PM (afternoon)
24 hours	21.00	29.00	25.00	87.00	72.00	85.00
48 hours	22.00	29.00	23.00	82.00	62.00	84.00
72 hours	20.00	27.00	24.00	87.00	77.00	85.00
96 hours	22.00	31.00	25.00	82.00	66.00	78.00
120 hours	19.00	30.00	25.00	81.00	62.00	84.00
144 hours	21.00	29.00	23.00	80.00	74.00	86.00
168 hours	22.00	29.00	25.00	85.00	67.00	85.00
Mean ± Std. Dev	21.00a ± 1.15	29.14c ± 1.21	24.29b ± 0.95	83.43b ± 2.88	68.57a ± 5.88	83.86b ± 2.67
Std. Error	0.44	0.46	0.36	1.08	2.22	1.01
Lower Bound (95% Confidence Interval for Mean)	19.93	28.02	23.40	80.76	63.13	81.28
Upper Bound (95% Confidence Interval for Mean)	22.07	30.27	25.18	86.09	74.01	86.33

Note: Numbers in rows and columns followed by the same letter indicate no difference at the 5% significant level (α) according to Tukey Test

Environmental Abiotic Factors

Temperature and humidity at the time of observation, fluctuated every hour of observation. The daily average temperature at the 24th to 168th hour of observation has a temperature range of 19–22°C at morning 07.00 am, range of 27–31°C at noon 12.00 pm, and range of 23–25°C at the afternoon. While the relative humidity (RH) has a RH range of 80–87% at morning 07.00 am, range of 62–72% at noon, and range of 78–86% (Table 7). This result is a little different from the other research, that have shown the rice bug pests like areas with warm weather with optimum temperatures ranging between 25.30-26.75°C and relative humidity ranging from 73.75–74.92% (Triaswanto et al., 2019). However, the other research said that the best to apply biopesticides when the temperature is not high because high temperatures (>60°C) can change and damage the phytochemical content of biopesticides (Ocktaviani et al., 2018). Meanwhile, insects are poikilothermic organisms where the body temperature will depend on the temperature of the environment where it live so that temperature is an important factor in it's development. Insects have a physiology that is sensitive to changes in temperature, where low temperatures in winter or rainy season in field can increase the mortality of insect pests and reduce their population numbers (Skendžić et al., 2021). The body temperature of rice bug can change according to the environmental temperature, but if the environmental conditions are too extreme, the animal can stress and even die (Taradipha et al., 2019).

Conclusion

The extract has potential as a biopesticide with yield value 8.3 – 12; pH value 4.84 – 5.37; and positive secondary metabolite. Meanwhile, toxicity tests showed the highest mortality at concentration 50%, LC50 at 144th hours and 168th hours was 4,373 and 2,002. Besides that, biopesticides changes morphology and behavior pest. Observations abiotic factors showed that rice bug can survive in temperature range of 22.75 – 25°C and relative humidity of 77 - 84%. In the future, it hoped biopesticides can be commercialized to reduce dependence on chemical pesticides.

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Conceptualization, F.W.L.; methodology, F.W.L; D.M; Z.P.G.; software, F.W.L.; validation, D.M; Z.P.G.; formal analysis, F.W.L.; investigation, F.W.L.; resources, F.W.L; D.M; Z.P.G.; data curation, F.W.L; D.M; Z.P.G; writing—original draft preparation, F.W.L; review, D.M; Z.P.G; visualization, F.W.L; supervision, D.M; Z.P.G.; project administration, F.W.L.; funding acquisition, F.W.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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