Efficacy of Red Betel Leaf’s (*Piper crocatum*) Chloroform Extract as Repellent Against Rice Bugs *Leptocorisa acuta* Thunberg, 1783 (Hemiptera: Alydidae)

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Abstract. Rice bugs *Leptocorisa acuta* is one of the insects that can damage and reduce rice production by sucking the sap inside the rice grains during the milky phase. The usage of red betel leaf’s (*Piper crocatum*) extract as bio repellent is one of the alternatives to reduce rice bug’s pest without causing any environmental degradation. This research aims to compare and determine the most effective concentration level of red betel leaf’s chloroform extract as a repellent. The red betel leaf extract was tested at concentrations 25%, 37.5%, and 50%. The methods in this research included sampling and acclimatization of rice bugs and repellency test of rice bugs using various concentrations of the extract. The study results were analysed using Probit Analysis with Lethal Concentration (LC₅₀) according to the number of rice bug’s repellency. The results showed that at 59.75% concentration of red betel leaf extract’s chloroform effectively repelled 50% of the rice bug’s population and reduced the number of rice bugs population below the economic threshold level.

1. Introduction

Indonesia is an agricultural country, where the main production comes from the agricultural sector. Rice is the main agricultural product in Indonesia [1]. There are many obstacles in the rice production sector, one of those is insect pests that could damage the rice plants.

Rice bugs *Leptocorisa acuta* Thunberg, 1783 is one of the potential agricultural pests that can damage rice plants by sucking the grains during the milky phase using their rostrum. As a result, rice grains are not filled or even empty [2]. Rice bugs, both nymph, and imago, not only suck the grains of rice during the milky phase but also suck the liquid of rice stalks. In a situation where there are no ripe milky phase grains, rice bugs still can suck the rice grains, which start to harden by releasing enzymes that can digest carbohydrates [3].

Based on research conducted by the Indonesian Center for Rice Research, shows that a rice bugs population of five individuals in nine clumps of rice can reduce agricultural production by 15%. The relationship between rice bugs’ population density and the decrease in production shows that one individual rice bug in one panicle in one week can reduce agricultural production by 27% [4]. To control rice bugs population which is often carried out by using artificial insecticides to kill them, can impact
the environment because it is difficult to degrade and leaves a residue, so it can increase environmental pollution and insect’s resistance against chemicals [5].

One of the biological products known to be safe for the environment and human health is a natural insecticide made from plant materials that can inhibit growth and development or kill pests and organisms that could cause a disease. Plants contain chemicals as secondary metabolites, where these compounds have an important role in the process of interacting or competing, including protecting themselves from the interference of their competitors [6]. These secondary metabolite products can be used as active ingredients of bio-pesticides [7,8] and used by plants as a defense mechanism against invading organisms. Bio-pesticides have organic compounds that are easily degraded in nature [9].

One of the potential plants as bio-insecticide is from a group member of the Piperaceae family, which contains essential oils as a repellent [10]. Betel leaf contains chemicals such as pyrimidines, alkaloids, tannins, saponins, flavonoids, and polyphenols. Three piperamide compounds from betel leaf, such as piperside, dehydropiperside, and guininsin have a contact effect and can cause immediate knockdown effects [10]. The piperamide compound works as a neurotoxin by disrupting nerve impulse’s delivery to central nerve axons, causing insects to become paralyzed and eventually die [11]. This research was carried out to know the efficacy of red betel chloroform extract, which potentially as a repellent against rice bugs.

2. Materials and Methods

The materials used in this study include rice bugs *L. acuta*, chloroform extracts of *P. crocatum* leaves with concentrations of 25%, 37.5%, and 50%, and the milky stage of rice plants (*Oryza sativa*). The tools used in this study include a sweep net and modified glass box olfactometers as a place to treat and test the rice bugs.

Samples of rice bugs were taken in the rice fields at Jalan Palagan, Yogyakarta, using a sweep net, and then rice bugs were put in a jar with a small hole and a plastic lid. After that, rice bugs were kept in the Entomology Laboratory, Faculty of Biology, Gadjah Mada University, Yogyakarta. The sampling results of rice bugs were put in a gridded box in which the cooked rice milk had been added. Then, it was left for a few days for acclimatization to adapt to the laboratory environment.

The repellent test was carried out using 3 glass boxes as an olfactometer. In a glass box, 5 to 6 panicles of milky stage rice plants were added. At the top of the glass box, the cover used for the glass box was replaced using a thick cardboard box measuring 37.5 x 30 x 37.5 cm with the top, bottom, front, and back perforated so that only about 3 cm was left on the edges. Then, the part that was perforated was covered by a clear plastic cover so that light could enter the cardboard box. At the bottom of the cardboard, a hole was made in the middle of the clear plastic cover with a diameter of about 7 cm as a path between the cardboard box and the glass box. On the top surface of the cardboard box, a small hole to insert the oxygen hose was made. In the glass box, a hole was made at the upper part with a diameter of about 2 cm to insert the rice bugs and spray the extracts. Then, as many as 10 rice bugs were put into the glass box. After that, the hole was closed first. Then, in the first glass box, a 25% concentration of red betel leaf extract mixed with DMSO and distilled water was sprayed to the rice plants inside the glass box, and the hole closed. It was left for 2 days with an observation range of 24 and 48 hours. Then, in the same way, red betel leaf extract concentrations of 37.5% and 50% were tested.

Data analysis was performed using Microsoft Excel 2010, and calculations were carried out using Probit Analysis with Lethal Concentration (LC₅₀) to determine the most effective concentration of red betel leaf’s chloroform extract as a repellent to the rice bugs.

3. Result and Discussion

The red betel leaf extract concentration tested on rice bugs includes concentrations of 25%, 37.5%, and 50%. The result indicated that there was a repellent response from 10 rice bugs tested at each concentration. The number of rice bugs repelled at the lowest concentration of 25% red betel leaf chloroform extract was 4. At 37.5% red betel leaf chloroform extract concentration, the number of repelled rice bugs was 4. Whereas, at the highest concentration of 50% red betel leaf chloroform extract,
the number of repelled rice bugs was 5. The highest number of rice bugs that were repelled was at the 50% concentration of red betel leaf chloroform extract with the highest percentage of repelled response, up to 50% from the total number of rice bugs tested in the sample. The Lethal concentration \( (LC_{50}) \) value obtained from these data was 59.75%.

Table 1. The ratio between \textit{Piper crocatum} chloroform extract concentration and number of tested \textit{Leptocorisa acuta}

| Extract Concentration | Tested \textit{L. acuta} (n) | Number of \textit{L. acuta} | Repelled Percentage | Lethal Concentration \( (LC_{50}) \) |
|-----------------------|-------------------------------|-----------------------------|---------------------|-----------------------------|
| 25%                   | 10                            | 4                           | 0                   | 4                           | 40%                        |
| 37.5%                 | 10                            | 4                           | 2                   | 2                           | 40%                        | 59.75%                     |
| 50%                   | 10                            | 5                           | 5                   | 3                           | 50%                        |

The linear regression equation from the data analysis (Table 1) is \( y = 0.75x + 3.65 \). It indicates that the higher concentration of red betel leaf extract used to cause a higher mortality rate of the rice bugs. The Lethal Concentration \( (LC_{50}) \) value obtained from the equation formula was 59.75%. This value implies that 59.75% red betel leaf extract could repel 50% of the total number of tested rice bugs.

Essential oil is a secondary metabolite of the red betel leaf extract that acts as a repellent. The aromatic compounds of essential oil interact with the central nervous system by stimulating the olfactory system in insect antennae and brain nerves below the cerebral cortex balance [12]. Locomotor activity is movement activity resulting from electrical activity change after the postsynaptic permeability membrane shifts and a presynaptic neuron in the central nervous system releases the transmitter to block the lactate receptor. Insects will avoid the smell of essential oils after those mechanisms happen [13]. Essential oils contain terpenoids, hydrocarbons, and aromatic compounds. Terpenoid contains substances that work as a repellent, such as cineol, eugenol, limonene, terpinolene, citronellol, camphor, and timol [14].

Boonde in Mulyantama [15], stated that the increasing concentration of the extract could bring up the aromatic compounds that are not liked by insects. This was because the repellent rate of rice bugs increases continuously at high concentration extract. Apart from the repellent effect, the rice plants containing aromatic compounds were also found to be more protected from rice bugs and toxic for insects. According to Scott et al. (2008), Piperimida compounds, which can be found in various \textit{Piper} plants, play a role as active compounds and work as a neurotoxin resulting in knockdown effect or rapid insect mortality [11]. This was evidenced by the observation obtained in 24 hours. Besides the presence of repellent activity, the rice bug’s mortality was also indicated after the rice plants were sprayed with various concentrations of red betel leaf chloroform extract.

In this study, further calculation was carried out to know the repellent ability of red betel leaf chloroform extract to suppress rice bugs population in the economic threshold level. Purnomo [16] from Riau Province Department of Agriculture stated that the economic threshold level of rice bugs (\textit{Leptocorisa} spp.) according to their past research, there were 5 individuals in 1 m² field. Five rice bugs in nine clumps of rice plants can reduce rice yields to 15% [17]. There are around 160.000 rice plant clumps with the planting space of 25 cm x 25 cm between each paddy in one hectare of rice fields [18]. The repellency ability of red betel leaf chloroform extract in reducing the rice bugs population based on the calculation is shown in Table 2.
Table 2. Calculation of Rice Bugs Population

| Rice Bugs Population in Economic Threshold Level | Rice Bugs Population Reduce 15% Rice Yields |
|-------------------------------------------------|------------------------------------------|
| 5 ind / 1 m²                                    | 5 individual / 9 rice plants clumps      |
| 1 ha = 10,000 m²                                | 1 ha = 160,000 paddy clumps             |
| (planting space between clumps 25 cm x 25 cm)   |                                          |

→ 5 ind x 10,000 m² = ± 50,000 rice bugs in 1 ha of rice field

→ \( \frac{5}{9} \times 160,000 = \pm 88,889 \) rice bugs in 1 ha of rice field (9 ind / 1 m²)

It was known that before the application of red betel leaf chloroform extract, the number of rice bugs population in economic threshold level is ± 50,000 rice bugs in 1 ha of rice field, while the estimated rice bugs population could reduce rice production by 15% is ± 88,889 rice bugs in 1 ha of a rice field (9 ind/ 1 m²). So, rice bugs could reduce 15% of rice production if the number of rice bugs populations exceeds the number of rice bugs in the economic threshold level.

In this study, it was found that the total population of rice bugs at the economic threshold level before the application of red betel leaf chloroform extract is ±50,000 rice bugs in 1 ha of a rice field. Meanwhile, the estimated rice bug population, which could reduce around 15% rice production is ± 88,889 rice bugs in 1 ha of a rice field (9 ind/ 1 m²). It said that the rice bugs could reduce the 15% of rice production if the number of rice bugs population exceeds the economic threshold level.

Applying red betel leaf chloroform extract at a concentration of 59.75% could reduce 50% of the rice bugs population. The pre-estimation showed that ±88,889 rice bugs in 1 ha of rice fields (9 ind/ 1 m²) that exceeds the economic threshold level could reduce 15% of the rice production, then by giving the red betel leaf chloroform extract at a concentration of 59.75% will be able to reduce ±44,445 rice bugs number in 1 ha of a rice field (4 individual/ 1 m²). This concentration can be declared effective in reducing 50% of the rice bugs population in a rice field where this number is still below the economic threshold level, and it can minimize the decline in rice production.

4. Conclusion

Based on this research, we concluded that red betel leaf extract’s chloroform effective as bio-repellent with the 59.75% extract concentration could repel 50% of the population of the rice bugs and effectively reduce the number of rice bugs’ population below the economic threshold level.

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