Repellent Activity of Waste Extract from Two Local Medicinal Plant Against Rice Weevil (Sitophilus oryzae)

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Repellent Activity of Waste Extract from Two Local Medicinal Plant Against Rice Weevil (*Sitophilus oryzae*)

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Amomum cardamomum; Zingiber zerumbet; Sitophilus oryzae; Waste Extract; Repellent

Abstract

In order to find natural and inexpensive bioinsecticides, the active compound of waste extract from two medicinal plants were analyzed. Ethanolic extracts of *Amomum cardamomum* and *Zingiber zerumbet* were evaluated under laboratory conditions for their repellency against rice weevil (*Sitophilus oryzae*, L.). Four extracts concentration were tested in this research, i.e: 25, 50, 75 and 100%. Efficacy was measured based on the response of rice weevil in preference tests using Y Olfactometer Tube, then the results were converted into the Percentage Repellency (PR) and Preference Index (PI). The PR data were analyzed using ANOVA, whereas PI values were analyzed descriptively. The result showed that the PR were significantly (LSD test; $\alpha >$ 0.05) and revealed that waste extracts repel the rice weevil at 100% solution. The highest PR was found at 100% solution in both extracts, however, statistically the repellency of *A. cardamomum* waste extract was higher than *Z. zerumbet*'s. Likewise, the PI of both extracts showed negative value at 75 and 100% concentration. This study concluded that extract of *A. cardamomum* and *Z. zerumbet* wastes have repellent effect against rice weevil and *A. cardamomum* repellency is better than *Z. zerumbet.* The findings of this study can provide a useful information for development of a rice weevil repellent extracted from waste product of medicinal plants that is more economically and environmentally friendly than synthetic insect repellents.

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INTRODUCTION

Rice weevil (*Sitophilus oryzae* L.) is the main pest that causes damage to rice in storage. Adult rice weevil consumes and causes the damage to rice from the outside, while the larva eats away nutrients from the inner grain of rice. Generally, control of rice weevil and other insects in the storage is performed using the synthetic insecticides such as methyl bromide, phosphine, or sulfuryl fluoride by means of fumigation. Synthetic insecticide is effective because its toxicity and able to quickly kill the target pest. However, the application of synthetic pesticides has led to some problems including its toxicity and residual effects, environmental pollution, and resistance in insects. The use of chemical insecticides has a disadvantage because continuous use will adversely affect the ecosystem and human health (Arif, 2015). The synthetic insecticide leaves a residue that could potentially enter the body through inhalation, dermal exposure, and digestion. In line with government policy regarding organic farming systems that are stipulated in ISO 6729 (BSN, 2016), the use of synthetic insecticides is currently encouraged to be replaced or reduced. One of alternatives is the use of bioinsecticide that has the potential to develop is utilization the botanical insecticides.

Naturally, the plant contains an active component which acts as protection from insect pests. through various methods, including repellent, attracting, antifeedant, and toxicant (Hikal et al., 2017). According to Guswenrivo et al. (2013), active components such as saponins, alkaloids, flavonoids, triterpenoids and glycosides are able to provide the insecticidal effect. Plants that are potential as sources of bioinsecticide are generally comes from the medicinal plants (Hendrival & Melinda, 2017). Ethnobotany studies in many places of Java has been revealed that Zingiberaceae was found in many rural communities because this traditional medicinal plant still plays an important role in treatment of illnesses (Shanthi et al., 2014; (Malini et al., 2017). Most medicinal plants are from Zingiberaceae Family due to their high compositions of secondary metabolites such as alkaloid, saponin, tannin, and flavonoid (Hartanto et al., 2014).

According to the study by Afrina et al. (2016) and Wahyuni et al. (2013), that *Amomum cardamomum* and *Zingiber zerumbet* contain the active compounds that can be used as insecticidal. Both of them are from Zingiberaceae family and have became the main commodities of fragrance materials in pharmaceutical industry. That condition makes them economically unprofitable as a bioinsecticide material.

Most bioinsecticides or insect repellents products are generally produced using natural essential oils. In the harvest period, stems and leaves of both *A. cardamomum* and *Z. zerumbet* are often just thrown away, rarely used. Meanwhile, the stem and leaves (postharvest waste) are also known to contain the insecticidal compounds although only at low levels. It is indicated by the same smell, for example, essential oils. The "essential" term is used because essential oil represents the smell of the original plant (Mulyani, 2010). Silalahi (2018) found that essential oils of galangal plant *(Alpinia galanga* L.) are not only in the rhizome but in all organs of plants with varying concentrations. The results of the phytochemical analysis of various plants from Zingiberaceae showed the presence of essential oil content, flavonoids, alkaloids, steroids/terpenoids, and saponins in all organs (Tarigan et al., 2016). In an effort to find the source of affordable bioinsecticides for the control of rice weevil, this research was conducted to analyze the active compound contained in extracts of *A. cardamomum* and *Z. zerumbet* postharvest waste. This study will provide the information regarding the ability of *A. cardamomum* and *Z. zerumbet* to be used repellents against rice weevil.

METHODS

The study was conducted in January-April 2018. *A. cardamomum* and *Z. zerumbe* wastes were obtained from Temukencono herbal garden, Gunungpati, Semarang. Adult rice weevils were obtained from traditional markets. Then, they were cultured and tested in the laboratory of Biology, Universitas Negeri Semarang.

Insect Preparation

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) were used to determine repellent activities of *A. cardamomum* and *Z. zerumbet* waste extract. Adults rice weevils were reared on the rice in the laboratory at 28 ± 2°C, and 75±5% of humidity. Breeding (rearing) was done by putting 100 adults of male and female rice weevil into 10 jars, every jar was filled with rice as a medium for rice weevils maintenance. Every six days, rice was transferred to another jar, with the aim that the eggs left behind will hatch and obtained a relatively homogeneous with the same age range. The first generation of rice weevil was used for experiments.

Extract Preparation

Material samples from the post-harvest waste of *A. cardamomum* and *Z. Zerumbet* (stem and leaves) in a ratio of 1: 1 were washed, cut into pieces and dried under the shade. Dried samples were milled mechanically and stored in an airtight container. This powder was acerated in 96% ethanol with a ratio of 1: 1 to obtain 100% extract, and then the 100% extract was stored in a dark glass bottle. Some extracts were taken to be evaporated and analyzed for their active compounds contents using Gas Chromatography-Mass Spectrometry (GC-MS).

Phytochemical Analysis

Phytochemical analysis of extracts was conducted using GC-MS Perkin Elmer Method to determine the active compounds contained in the extract quantitatively (Guswenrivo et al., 2013). Active components obtained are shown in Tables and graphs.

Repellent Bioassay

Repellent bioassay of *S. oryzae* adults was studied using Y tube olfactometer which consists of three glass tubes as arms (10 cm length; 2 cm diameter). The end of each arm was fitted with a glass vial (3.5 cm length, 2 cm diameter). Arm A was as control, arm B was as treatment and arm C was as the entrance of the insect test. Concentrations of 25, 50, 75 and 100% of the extract were applied onto filter paper strips (2 cm diameter) and were allowed to dry for five minutes. The filter paper strips were then placed in the inner surface of the glass vials which were attached to the arms B of the olfactometer. Filter paper strip with ethanol was served as control (arm B). After the attachment of the glass vials to the arms of the olfactometer, twenty five adults rice weevil were entered into the olfactometer via arm A. After 1 hour of treatment, orientation of the direction was observed with the assumption that rice weevil will choose a safe passage for them, not disturbed by the sharp aroma of waste extracted placed in one of the glass vials. The number of insects that persist in arm A and B was counted. Each treatment was repeated five times. The number of adults found in each glass vial was recorded. The Preference Index (PI) and Percentage of Repellency (PR) were calculated using the formula of Seenivasagan et al. (2014); (Aryani & Auamcharoen, 2016).

$$PI = \frac{Nt - Nc}{Nt + Nc} \qquad PR = \frac{Nc}{Nc + Nt} \times 100\%$$

Nt: number of insects trapped in the crude extract or powder test chamber

Nc: number of insects trapped in the control test chamber

Data of PI is presented in the form of a graph and is analyzed descriptively. If the PI value is negative, it means there is a repellent effect to insect (Jayakumar et al., 2017). The PR is presented in the table of PR ± SD averages. Data of PR was analyzed using the analysis of variance (ANOVA) and continued with LSD test ($\alpha < 0.05$). The percentage of repellency was also converted into criteria according to (Rinaldi et al., 2016) namely :

PR value <20% ; no repellent

20% ≤ PR <40% ; low repellent

40% ≤ PR <60% ; medium repellent

60% ≤ PR <80% ; high repellent

PR ≥ 80 %: very high repellent

RESULTS AND DISCUSSION

Identification of active compounds using GC-MS showed that waste extract of *A. cardamomum* plant contained 4 major components, i.e. essential oils, fatty acids, saponins, and flavonoids. While, in the extract of *Z. zerumbet* plant, it was found the active compounds of essential oils, fatty acids, flavonoids, and alkaloids. All of them The graphs of the chromatograms of both extracts are shown in Figure 1 and Figure 2. These components are anti-insecticidal compounds according to Hikal et al. (2017).

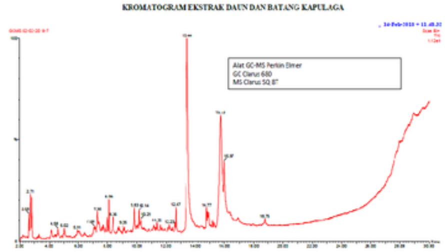

Figure 1. Chromatogram of waste extract from *A. cardamomum* plant

Both solutions were dark brown and each of them released a sharp odor like the aroma of *A. cardamomum* seeds and *Z. zerumbet* rhizome.

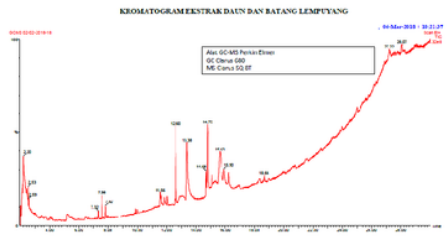

Figure 2. Chromatogram of waste extract from *Z. zerumbet* plant

Preference Index

Waste extract from *A. cardamomum* and *Z. zerumbet* were tested for their repellent activity against adults rice weevil at concentrations of 25, 50, 75 and 100%. In general, repellency increases with increase in concentration used in the treatment. The result of this study indicated variation among sources of extract and concentration. The preference index ranged from 0.54 to -0.15 (Figure. 1). PI expresses polarity of the directional/ orientation choice. Negative values indicate the repellent activity of extract against rice weevil.

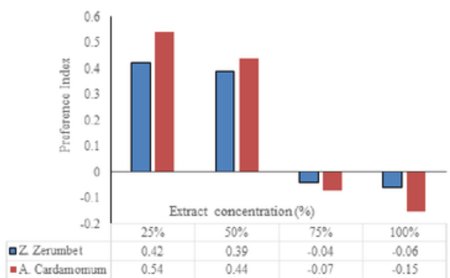

Figure 1. Mean value of Preference Index of *Z. zerumbet* and *A. cardamomum* waste extract

Rice weevil preferred to go to arm B (control) than to arm C (treatment), so that, the PI value becomes negative. Similarly sources the extract used also influenced the response of rice weevil (Figure 1). This condition is clearly related to the concentration of the active compound and the aromatic compound that comes out from the extract. The results of GC-MS analysis (Table 1) show the number of active components of essential oils, one of which is a compound of 1.8 Cineol which has been proven to be a repellent compound for various insects (Sainz et al., 2012); (Jemaa, 2014); (Niroumand et al., 2016).

At low concentrations (25% and 50%), the PI values of the two types of extract did not provide a repellent effect. In this treatment, the smell of extracts have not disturbed the olfactory system of test insects. On the other hand, at a concentration of 75% and 100%, the PI value was negative, indicating that the sensory organs of rice weevils detect any odor that disturbs them or that they dislike. According to (Jayakumar et al., 2017). Previous researchers have also proven that essential oils of various member of Zingiberaceae including *A. cardamomum* and *Z. zerumbet* have anti-insect effects (Abbasipour et al., 2011); (Amiri et al., 2016); (Korina & Habiyaremye, 2017); (Gopal & Benny, 2018). Thus, the two types of extracts had a repellent effect at a concentration of 75% and 100%, with the effect of *A. cardamomum* waste extract was greater than *Z. zerumbet*.

Percentage Repellency

Based on statistical analysis, it turns out that the difference in concentration has a significant effect on the percentage repellency (PR) of rice weevil ($\alpha < 0.05$). In contrast, differences in the source of extract did not significantly influence the PR. The results of the analysis also showed no interaction between the source and concentration of extract (Table 1). The data obtained were then further analyzed using LSD test, the results are shown in Table 2.

The average PR of *A. cardamomum* and *Z. zerumbet* extracts against rice weevil is presented in Table 2. Based on the repellency criteria according to (Rinaldi et al., 2016), extracts of *A. cardamomum* and *Z. zerumbet* at concentrations of 25% and 50% showed a low repellency effect (less than 50%). At 75% and 100% extract concentrations, the percentage repellency increased to more than 50% and was in the medium category. This increase occurred due to the change in orientation of the way leading toward increased control, particularly at concentrations of extracts 75% and 100%. In contrast, at low concentrations (25% and 50%), the number of rice weevil into the treatment less than 50%. Based on LSD- test ($\alpha < 0.05$) source and concentration of extract had a significant effect on the repellency. The highest percentage repellency is at a concentration of 100%, both in *A. cardamomum* and *Z. zerumbet* extracts. The concentrations of 75% and 100% is not different. Thus, extracts with a concentration of 75% are the most effective to be used in this study.

Phytochemical compounds contained in extract of each plant species have a repellency effect in accordance with the concentration level given. The more concentrated the extract, the higher the percentage repellency. This is in line with the results of the study by Abdelgaleil et al.

Table 1. The result of Two-way ANOVA test

Source of diversity	Number of squares	df	Middle Squares	F count	Sig.
SE	1.600	1	1.600	0.062 [ns]	0.806
CE	7049.600	3	2349.867	90.379 *	0.000
Interaction S E x Extract	155.200	3	51.733	1.990 [ns]	0.135
Error	832.000	32	26.000		
Total	8038.400	39			

Notes: * = significant, based on the LSD test at a significance level of 5%; ns = not significantly different; SE = Source of Extract; CE = Concentration of Extract;

Table 2. Results of LSD test on the percentage repellency of extracts against rice weevil (mean ± standard deviation)

Extract concentration (%)	Average repellency (%)	
	A.Cardamomum	Z. zerumbet
25	23.20 ± 5.93[a]	28.80 ± 7.69[a]
50	28.00 ± 2.82[a]	30.40 ± 4.56[a]
75	53.60 ± 4.56[b]	52.00 ± 5.67[b]
100	57.60 ± 4.56[b]	52.80 ± 3.34[b]
Average	40.60 ± 16.11[ns]	41.00 ± 12.77[ns]

Notes: -The means followed by a different letter in the same column are significantly different (LSD; $\alpha < 0.05$); -The means followed by a different letter in the average row are significantly according to student t-test ($\alpha < 0.05$).

(2016) which stated that phytochemical compounds have an insecticidal effect depending on the dose given. Essential oils contained in *A. cardamomum* extract provide a repellency effect on rice weevils that are more dominant than other compounds. In line with the previous research states that essential oils have a repellent effect on *Tribolium castaneum* rice infestation. Fatty acids are also included in insecticidal compounds (Hikal et al., 2017). In this study, fatty acids are one of the dominant elements in the extract, so it is suspected that fatty acids also contribute in providing a repellent activity against rice weevil. In this study, the concentration of 100% waste extract between 52.8% and 57.6% so that can say the repellent effect is in the medium category (Rinaldi et al., 2016). According to the standards of the Indonesian Pesticide Commission, repellent can be said to be effective if the average protection power reaches 90% (Korneliani, 2011). Therefore, there is possibility to increase the effectiveness of the extract by increasing the concentration level more concentrated or increasing the dose. The findings of this study can provide a useful information for development of a newer rice weevil repellent extracted from waste-product of medicinal plants. This repellent can be an alternative that is more economically and environmentally friendly than synthetic insect repellents.

CONCLUSION

This study concluded that *A. cardamomum* and *Z. zerumbet* waste extracts have repellent activity against rice weevil. Repellency of *A. cardamomum* was higher than *Z. zerumbet.*

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