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SUMMARY

REVIEW

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Submission

Authors	Priyantini Widiyaningrum, Devy Candrawati, Dyah Rini Indriyanti, Bambang Priyono
Title	Repellent Activity of Waste Extract from Two Local Medicinal Plant Against Rice Weevil (<i>Sitophilus Oryzae</i>)
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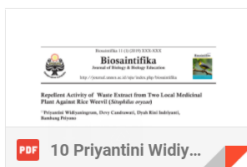
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Repellent Activity of Waste Extract from Two Local Medicinal Plant Against Rice Weevil (*Sitophilus Oryzae* L.)

Priyantini Widiyaningrum, Devy Candrawati, Dyah Rini Indriyanti, Bambang Priyono

Department of Biology, Faculty of Mathematics and Natural Sciences

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ABSTRACT

In order to find natural and ~~cheaper-inexpensive~~ bioinsecticides, the active ~~components-compound~~ of waste extract from two medicinal plants were analyzed. Ethanolic ~~plant~~ extracts of *Amomum cardamomum* and *Zingiber zerumbet* were evaluated under laboratory conditions for their repellency against rice weevil (*Sitophilus oryzae*, L.). Four extracts ~~solution-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 ~~Ppercentage of #R~~ Repellency (PR) and Preference Index (PI). The ~~percentage-of-repellencyPR data~~ were analyzed using ANOVA, whereas ~~preference-indexPI~~ values were analyzed descriptively. The result showed that the ~~percentage-of-repellencyPR~~ were significantly (LSD test; $\alpha > 0.05$) and revealed that waste extracts repel the rice weevil at 100% solution. ~~The highest percentage-of-repellencyPR~~ was found at 100% solution in both extracts, however, statistically the repellency of ~~extracting Amomum-A. cardamomum~~ waste ~~extract~~ was higher than ~~Z.ingiber zerumbet's~~ waste. Likewise, the ~~preference-indexPI~~ of both extracts showed negative value at 75 and 100% ~~solutionconcentration~~. This study concluded that extract of *A. cardamomum* ~~Amomum cardamomum~~ and *Z.ingiber zerumbet* wastes have repellency effect against rice weevil and, but *A. cardamomum* repellency ~~cardamom waste~~ is better than *Z. zerumbet*. ~~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. ~~The findings of this study can be positive information for people, to develop a newer anti rice weevil repellent from herbal waste based product, as an alternative repellent that is cheaper and environmentally friendlyeee friendly-~~ than synthetic insect repellents.

Keywords: *Amomum cardamomum*, *Zingiber zerumbet*, *Sitophilus oryzae*, waste extract, repellent.

INTRODUCTION

~~The rice~~Rice weevil (*Sitophilus oryzae* L.) is the main pest that causes damage to rice in storage. Adult rice weevil consumes and ~~causes the~~ damage ~~of-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 ~~it-is~~its toxicity and ~~able to~~ quickly kill~~s~~ 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

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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 reduced-replaced or reduced. One of alternatives is the use of bioinsecticide that has the potential to develop is utilization the botanical insecticides.

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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), an active components such as saponins, alkaloids, flavonoids, triterpenoids and glycosides giving are able to provide the insecticidal effect. Plants that are potentially as a sources of bioinsecticide (Hendriyal & Melinda, 2017), are generally comes, among others are from the herbal medicinal plants. Etnobotany- studies in many places of Java it-has been revealed that Zingiberaceae was found in many rural communities because this traditional herbal medieinmedicinal plant has-still played-plays an important role in treatment of illnesses (Shanthi et al., 2014; (Malini et al., 2017). Most medicinal plants are from The Zingiberaceae Family contributes to the highest number of medicinal plants, because probably be due to their high compositions of secondary metabolites- such as alkaloid, saponin, tannin, and flavonoid (Hartanto; et al., 2014).

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Amomum cardamomum and Zingiber zerumbet are two of the thousands of plants in the family Zingiberaceae. According to the study by (Afrina et al., (2016) and Wahyuni et al., (2013), stated that Amomum cardamomum and Zingiber zerumbet contain the active compounds which are that can be used as insecticidal. Both of them are from Zingiberaceae family and have became the main commodities of seasoning-fragrance materials and in the pharmaceutical industry. That condition makes them economically unprofitable, so economically it's not profitable as a bioinsecticide material.

Field Code Changed

Field Code Changed

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 plants are often just thrown away, rarely used. Meanwhile, the stem and leaves (postharvest waste) are of the plants-also known to contain the insecticidal compounds although only at low levels. This is characterizedIt is indicated by the same aromasmell, for example, essential oils. The "essential" term is used because eEssential oil represents the smell of the original plant (Mulyani, 2010). (Silalahi, (2018) found that essential oils of galangal plant (Alpinia galanga L.) are found-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 plants-showed the presence of essential oil content, flavonoids, alkaloids, steroids/terpenoids, and saponins in all organs (Tarigan et al., 2016). In an

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effort to find the source of ~~cheap-affordable~~ bioinsecticides for the control of rice weevil, this research was conducted to analyze the active compound ~~contained in from waste~~ extracts of *A. momum* cardamomum and *Z. ingiber* 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. The findings of this study will inform to farmers that waste extract herbal can be used as the simple repellent on the rice weevil.~~

METHODS

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

Insect Preparation

The rice weevil, *Sitophilus* ~~S.~~ *oryzae* L. (Coleoptera: Curculionidae) were used to determine repellent activities of ~~waste extract from A. Cardamomum~~ and *Z. Zerumbet* ~~waste extract~~. Adults rice weevils were reared on the rice in the laboratory at $28 \pm 2^\circ\text{C}$, and $75 \pm 5\%$ of humidity. Breeding (rearing) ~~is was~~ done by ~~investing putting~~ 100 adults of male and female rice weevil into 10 jars, ~~each every of jar which is 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 ~~in under~~ the shade. Dried samples were milled mechanically and stored in an airtight container. This powder was macerated in 96% ethanol with a ratio of 1: 1 to obtain 100% extract, and then ~~the extract~~ 100% ~~extract~~ was stored in a dark glass bottle. Some extracts were taken to be evaporated and analyzed ~~for its- their active compounds~~ contents ~~of the active compounds~~ using Gas Chromatography-Mass Spectroscopy (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. sitophilus* *oryzae* adults was studied using Y tube olfactometer (Figure 1) which consists of three glass tubes ~~as arms~~ (10 cm length; 2 cm diameter). The end

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of each ~~glass~~ 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 ~~waste~~ 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 ~~on~~ in the inner surface of the glass vials which were attached to the arms B of the olfactometer. Filter paper strip ~~which receives with~~ ethanol ~~only was~~ served as control (arm B). After the attachment of the glass vials to the arms of the olfactometer, twenty five adults ~~of~~ rice weevil were ~~introduced~~ entered into the olfactometer via arm A. After 1 hour of treatment, orientation of the direction ~~is 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~~ et al., (2014); (Aryani & Auamcharoen, 2016).

$$PI = \frac{Nt - Nc}{Nt + Nc}$$

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

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~~ et al., 2017). The PR is presented in the table of PR \pm SD averages. ~~Data of PR was and~~ analyzed using the analysis of variance ~~of~~ (ANOVA) and continued with further LSD test ($\alpha < 0.05$). The percentage of repellency was also converted into criteria according to (Rinaldi ~~et al~~ et al., 2016) namely :

1. PR value <20% ; no repellent
2. 20% \leq PR <40% ; low repellent
3. 40% \leq PR <60% ; medium repellent
4. 60% \leq PR <80% ; high repellent
5. PR \geq 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

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chromatograms of both extracts are shown in Figure 1 and Figure 2. These components are anti-insecticidal compounds according to (Hikal ~~et al.~~ (2017).

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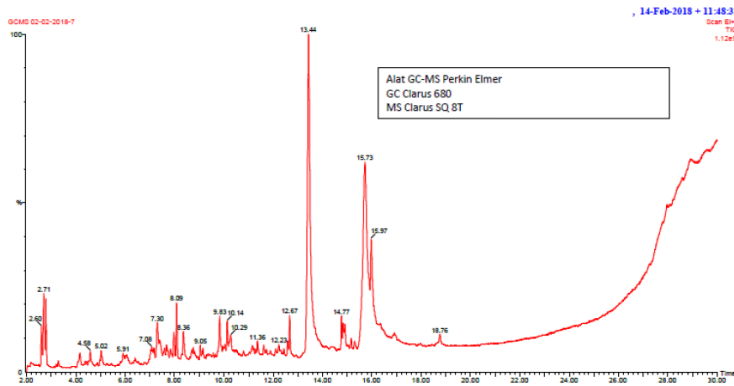


Fig 1. Chromatogram of waste extract from *A. cardamomum* plant

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

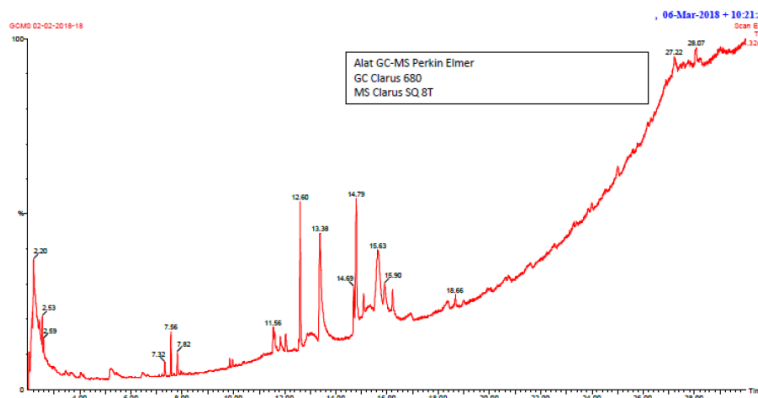


Fig 2. Chromatogram of waste extract from *Z. zerumbet* plant

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Preference Index

~~Two different waste~~Waste extract from *A. cardamomum* and *Z. Zerumbet* ~~plant was~~ were tested for their repellent activity against adults ~~of~~rice weevil at concentrations of -25, 50, 75 and 100% ~~for repellent activity.~~—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 23.20 to -0.15 (Fig. 3). Preference index (PI) expresses polarity of the directional/orientation choice. Negative values indicate the repellent activity of extract against rice weevil.

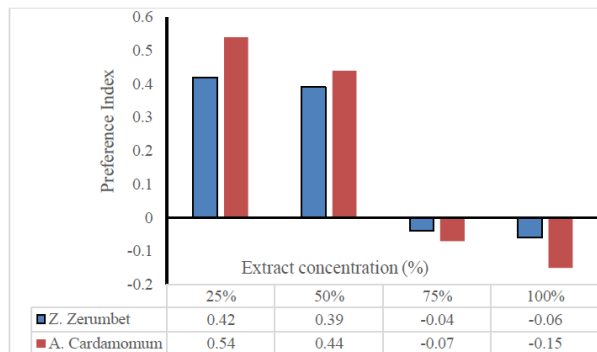


Figure 1. Mean value of Preference Index of waste extract from *Z. Zerumbet* and *A. cardamomum* waste extract plant

This is due to the directional orientation of rice weevil preferred to more go to arm B (control) compared than to arm C (treatment), so that the preference index (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 preference index (PI) values of the two types of extract did not provide a repellent effect. In this treatment, the aroma-smell of extracts has not disturbed the olfactory system of test insects. On the other hand, at a concentration of 75% and 100%, the PI value is was negative, indicating that the sensory organs of rice weevils detect any aroma-odor that disturbs them or that they dislikes it. According to (Jayakumar *et al.*, 2017), the negative on preference index value is an indicator that insects respond to the odor of aromatic other active compounds detected by their sensory organs. Previous researchers have also proven that essential oils of various member of Zingiberaceae plants including *A. Cardamomum* and *Z. zerumbet* have anti-insect effects (Abbasipour *et al.*, 2011); (Amiri, *et al.*, 2016); (Korina & Habiyaemye, 2017); (Gopal & Benny, 2018). Thus, the two waste-types of extracts had a

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repellent effect at a ~~treatment~~ concentration of 75% and 100%, ~~but with an 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 ~~of~~ repellency (PR) of rice weevil ($\alpha < 0.05$). In contrast, differences in the source of ~~waste extract~~ extract did not significantly influence the ~~percentage of repellency~~ PR. The results of the analysis also showed no interaction between the ~~extracted source~~ source and concentration of extract ~~and extract concentration~~ (Table 1). The ~~data obtained were then further analyzed using~~ LSD ~~further test, the~~ results are shown in Table 2.

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. ~~Further Results of LSD testing test of LSD~~ on the percentage ~~of~~ repellency ~~on of~~ extracts against rice weevil (mean \pm standard deviation)

Extract concentration (%)	Source of extract	
	<i>A. Cardamomum</i>	<i>Z. zerumbet</i>
25	23.20 \pm 5.93 ^a	28.80 \pm 7.69 ^a
50	28.00 \pm 2.82 ^a	30.40 \pm 4.56 ^a
75	53.60 \pm 4.56 ^b	52.00 \pm 5.67 ^b
100	57.60 \pm 4.56 ^b	52.80 \pm 3.34 ^b
Average	40.60 \pm 16.11 ^{ns}	41.00 \pm 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 ($\alpha < 0.05$).

The average ~~percentage of repellency~~ PR of *A. cardamomum* and *Z. Zerumbet* extracts ~~rice weevil~~ against ~~rice weevil~~ extracts of *A. cardamomum* and *Z. Zerumbet* waste

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Average repellency (%)

is presented in Table 2. Based on the ~~repellency rejection~~-criteria according to (Rinaldi ~~et al~~ al., 2016), extracts ~~waste~~ of *A. Cardamomum* and *Z. zerumbet* ~~plant~~ at concentrations of 25% and 50% ~~gave showed~~ a low ~~repellency rejection~~ effect (~~because they were at a percentage of less than 50%~~). At 75% and 100% extract concentrations, the percentage ~~of~~ 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 ($\alpha < 0.05$) ~~each source and concentration of~~ extract had a significant effect on the ~~percentage of~~ repellency, ~~with the concentration of extract showing significant differences with~~ ~~The highest percentage repellency of rejection 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 ~~optimum effective to be used~~ in this study.

Phytochemical compounds contained in extracts of each ~~waste plant species~~ have a ~~rejection repellency~~ effect in accordance with the concentration level given. The more concentrated the extract ~~was~~, the higher the percentage ~~repellency of rejection~~. This is in line with the results of the study ~~of by~~ Abdelgaleil ~~et al~~ al. (2016) which ~~states stated~~ that phytochemical compounds have an insecticidal effect depending on the dose given. Essential oils contained in *A. cardamomum* ~~cardamom~~ extract provide a ~~repellency rejection~~ effect on rice ~~mites 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~~ 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 ~~to in~~ providing a repellent activity ~~on 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~~ 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, ~~it is still very possible to increase it~~ by increasing the concentration level more concentrated or increasing the dose. The findings of this study can ~~be positive provide a useful~~ information ~~for people, to develop for development of~~ a newer anti-rice weevil repellent ~~extracted from herbal waste based product waste product of medicinal plants. This repellent can be, as~~ an alternative repellent that is ~~more cheaper and eco friendly economically and environmentally friendly~~ than synthetic insect repellents.

CONCLUSION

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Provide the citation

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This study concluded that ~~extracts of *A. cardamomum* and *Z. zerumbet* waste~~ ~~extracts~~ have repellent activity against rice weevil, ~~but the r~~Repellency of ~~extracting *A. cardamomum* waste~~ was higher than *Z. zerumbet* waste. ~~The findings of this study can be positive information, that waste extracts of *A. cardamomum* potentially as a repellent to rice weevil that is cheaper and easily applied.~~

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The author would like to thank Semarang State University for funding this research, through the 2018 UNNES DIPA fund with Number: SP DIPA-042.01.2.400899 / 2018.

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