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Insecticidal effect from waste extract of two local spices plant on the rice weevil

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Abstract. In an attempt to found natural and cheaper methods for the control of rice weevil, waste extract from two local spices plant were evaluated. Ethanol extracts (mix of leaf and stem parts) of *Zingiber zerumbet* and *Amomum cardamomum* were tested on rice weevil for their insecticidal effect. The rice weevil was treated under four different exposure times (0, 1, 2, and 3 hours) and then maintained for 3 weeks in the rice storage. The difference in weight loss of rice (WL) was assumed as feed consumption, and it was an indicator of the insecticidal effect. Percentage of WL were analyzed using ANOVA. The results showed that exposure time was significant to WL on both extracts (LSD test; $\alpha < 0.05$). The insecticidal effect with the lowest WL of rice occurred in a 3-hour treatment. The percentage WL in *A. cardamomum* is lower than those in the *Z. Zerumbet*. In conclusion, both plants' waste extract decreased the feeding activity on the rice weevil, but the waste extract of *A. cardamomum* is more effective than *Z. zerumbet*. This study demonstrates that a waste extract of *A. Cardamomum* has potency as an insecticide for rice weevil, when appropriate formulation and application methods have been developed.

1. Introduction

Insect pests are the major problem in post-harvest storage products such as grains and rice throughout the world. They are very significant in reducing the quantity and quality of stored products. There are three important pest species from the genus *Sitophilus* spp. (Coleoptera: Curculionidae) Which damages the stored grain, that is *Sitophilus oryzae*, *Sitophilus granarius*, and *Sitophilus zeamais*. These insects eat grains, making them unfit for human consumption. This further reduces the selling power of food grains which causes huge losses for farmers. [1]. Tefera *et al.* [2] have estimated that rice and stored grain can be lost 30% to 50% during storage. Insects cause significant damage in the tropical area because of the gainful climatic conditions for their breeding during storage. Both larvae and adult, they are the cause of damage [3]. According to Tripathi [4], The larva grows and develops inside the seed, eating away at the seed, causing irregular holes in all the grain in which it is laid. Generally, the pest control on stored products still uses synthetic insecticides. Various kinds of chemical insecticides are widely available to eradicate pests in stored food products. However, chemical pesticides are expensive, ineffective, and harmful to the environment. Synthetic pesticides, if used continuously, can cause environmental problems, danger to human health, pollution, pests become resistant and disrupt the balance of the ecosystem. For example, synthetic insecticides cause residual toxic effects, environmental pollution, and insects' resistance [5-6]. Natural insecticide products have been developed and are well accepted because they have a series of biological



properties that are useful against pests. Active compounds derived from plants are proven to be used for pest control of stored products. The activity of insect pests is disrupted because this bioinsecticide has insect repellent properties, toxic effects, antifeedant properties, and inhibits insect growth. [6]. Plant-based formulations are recognized as an alternative to synthetic insecticides that are sustainable and eco-friendly because they are biodegradable. Researchers are focused on developing a bioinsecticide source to control insect populations. Secondary plant metabolites such as organic acids, alkaloids, terpenoids, saponins, and glycoalkaloids can be a tool for insect control, especially as a food ingredient protector. Previous studies have evidenced that plant chemical compounds having an insecticidal effect, and these secondary metabolites can be combined or partially be used in insect control in different ways. Chemical compounds derived from plants may be toxic, growth retardants, chemosterilants, antifeedants, repellents, or attractants. [7].

Antifeedant is called feeding deterrent, defined as chemicals compound that prevent feeding activity and not kill the insect directly. Eventually, the insect will die through starvation even though the food is available nearby. The plant compound inhibits feeding or interrupts insect feeding by making the food object unattractive or unpalatable [8]. Feeding deterrent means changing eating behavior. Secondary metabolites work by blocking feeding, directly impacting the peripheral sensilla (= sense organs) in insects. These active compounds also interfere with insects' eating behavior by affecting the central nervous system's work (after ingestion and absorption). At specific doses, it causes sublethal toxicity to insects. The antifeedant effect causes insects not to recognize food, refuse or avoid eating. According to Koul [9], the most accepted definition of feeding deterrent is phytochemicals compound, which deters insects from feeding after they have bitten the plant. Finally, they occur inhibition by gustatory responses.

Various plants from the Zingiberaceae family are known as ingredients for spices, flavorings, and medicines in Southeast Asia for their unique aroma and taste, and medicinal properties. Essential oils from the Zingiberaceae family, including *Zingiber zerumbet* (L) and *Amomum cardamomum* Wild, also have been investigated for their insecticidal activity against *Sitophilus* sp. [10]. Both species were perennial, which grows naturally in moist and shaded parts of lowlands or downhill. *Z. zerumbet* (L.) known locally in Indonesia as "lempuyang" or as "shampoo ginger" in English [11]. Post-harvest waste of *A. cardamomum* and *Z. zerumbet*, primarily the stems and leaves, is underutilized but can act as natural pesticides. Stems and leaves of *Z. zerumbet* and *A. cardamomum* plant are known to contain insecticide ingredients, although the percentage is not as large as those found on the rhizome. To find natural insecticides that are environmentally friendly and cheaper to control *Sitophilus oryzae* L., two post-harvest wastes of Zingiberaceae plants, namely *Z. zerumbet* and *A. cardamomum* plant, were evaluated in this study. This research was conducted to examine the ability of waste extracts of *Amomum cardamomum* and *Zingiber zerumbet* to control *Sitophilus oryzae*. The aspect observed was the repellency and feeding deterrent of both extracts.

2. Methods

This research is part of the development of bioinsecticides derived from waste materials. The trial was carried out at the Biology Laboratory of Semarang State University in the 2018-2019 period. Post-harvest waste of *Z. Zerumbet* and *A. cardamom* was collected from a garden owned by herbal farmers in Gunungpati, Semarang.

2.1. Extract preparation

Stem and leaf samples with a ratio of 1: 1) cleaned, chopped, and dried outdoors in the shade. The plants were dried for 2 days and finely powdered using a flour grinder. The powder was extracted with ethanol for 24 hours. A semi-solid extract was obtained after the complete removal of ethanol under low pressure. Extraction material is stored for preparation in trials. The GC-MS (Gas Chromatography-Mass Spectroscopy) method was used to analyze the content of the active compound.

2.2. Rice weevil culture.

Adult rice weevils were collected from traditional markets and then cultured to obtain F1. The insect cultures were maintained under control in the darkroom at $28\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ with a sex ratio of 1: 3 (male: female). Adult rice weevil from the breeding was used for these experiments.

2.3. Feeding deterrent test

To evaluate the feeding deterrence, some adult rice weevil were exposed to *Z. Zerumbet* and *A. cardamomum* extract in three levels for an exposure duration of 1, 2, and 3 hours. A dose of 100 μL of each extract was soaked separately on the filter papers Whatman (2 cm), put into a petri dish (10 cm), and as many as 25 adult rice weevils were put in and closed tight to create fumigation. Each treatment was fumigating in 1, 2, and 3 hours. Rice weevil that fumigation then transferred to the culture media, which had been filled with 10 g of rice. The culture media were covered with cotton cloths and tied with rubber bands as many 25 insects without fumigation treatment served as control. All treatments were incubated in the room at the same temperature and humidity. After 21 days, the remaining weight of rice for each box was calculated. The feeding deterrent effect was calculated based on rice's weight at the end of the treatment in percent (WL), followed by comparing WL data between treatments [12].

$$WL (\%) = \frac{(IW - EW)}{IW} \times 100$$

Notes: IW is the rice's weight at the initial experiment; EW is the weight of rice at the end of the experiment. The experiments were repeated five times.

2.4. Statistical analysis

The remaining weight of rice (WR %) is presented in the mean \pm standard error and analyzed using two-way ANOVA. Means were further testing by LSD-test when ANOVA was significant ($\alpha < 0.05$).

3. Results and discussion

3.1. The compound of waste extract.

The insecticidal constituents of many plant extracts are mainly fatty acids and some secondary metabolites. These results revealed components of plant extract of *A. cardamomum* and *Z. zerumbet* (Table 1).

Many secondary plant metabolites have solid biological activity against pests and have been commercial, including essential oil, terpenoid, flavonoids, alkaloid, and fatty acid compounds. The secondary metabolite components found in the waste extract of *A. cardamomum* (Table 1) were different than *Z. Zerumbet* (Table 2). However, Essential oils and some insecticidal compounds were detected on both extracts. This shows that volatile oil activity does not work alone, but generally because of the synergistic effect of all its compounds, as suggested by Ndomo. *et al.*[13]. In previous studies, it has been known that the Zingiberaceae class of medicinal plants contains secondary metabolites which are identified as insecticidal, in addition to being a source of traditional medicine [14]. Previous research [15] states that waste extract of *A. cardamomum* and *Z. zerumbet* have a repellent effect on *Sitophilus oryzae*. David *et al.* [16] said that phytochemical compounds mainly affect the mid-intestine and Malphigian tubules' epithelium in mosquito larvae. Besides, Maurya *et al.* [17] found evidence that the crude extract may be more effective than the active compounds' action individually. This natural synergy can prevent insect vector resistance.

Table 1. The GC-MS analysis in plant extract of *A. cardamomum*.

No.	Name of the Compound	Compound Nature	% Area
1.	<i>Benzene, 1-methyl-2-(1-methylethyl)-</i>	Volatile oil	2.33
2.	<i>Eucalyptol (1,8 Cineol)</i>	Volatile oil	1.06
3.	<i>Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α,3α,5α)-</i>	Volatile oil	0.96
4.	<i>Phenol, 2-methyl-5-(1-methylethyl)-</i>	Volatile oil	0.55
5.	<i>Ascaridole epoxide</i>	Volatile oil	0.79
6.	<i>7-Oxabicyclo[4.1.0]heptan-2-one, 6-methyl-3-(1-methylethyl)-</i>	Volatile oil	1.93
7.	<i>Icosapent</i>	Diterpenoid	1.20
8.	<i>1,3-Dimethyl-1-cyclohexene</i>	Aromatic compound	0.48
9.	<i>Cholestan-3-ol, 2-methylene-, (3β,5α)-</i>	Saponin	1.05
10.	<i>1-Heptatriacotanol</i>	Carotenoid pigment	0.59
11.	<i>Valencen</i>	Flavonoid	8.14
12.	<i>Globulol</i>	Flavonoid	0.98
13.	<i>2,5-Octadecadiynoic acid, methyl ester</i>	Fatty acid	1.52
14.	<i>Cyclopropanedodecanoic acid, 2-octyl-, methyl ester</i>	Fatty acid	1.02
15.	<i>Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-methyl ester</i>	Fatty acid	0.56
17.	<i>Hexadecanoic acid, methyl ester</i>	Fatty acid	1.40
18.	<i>n-Hexadecanoic acid</i>	Fatty acid	34.72
19.	<i>trans-13-Octadecenoic acid</i>	Fatty acid	30.51

Table 2. The GC-MS analysis in plant extract of *Z. Zerumbet*.

No.	Name of the Compound	Compound Nature	% Area
1.	<i>3,5-Dimethyl-[1,2]dithiolane 1,1-dioxide</i>	Sulfur compounds	1.02
2.	<i>Phosphoric acid, trimethyl ester</i>	Fatty acids	25.32
3.	<i>E-11-Methyl-12-tetradecen-1-ol acetate</i>	Fatty acids	1.47
4.	<i>Methyl palmitate</i>	Fatty acids	6.39
5.	<i>Palmitic acid</i>	Fatty acids	16.95
6.	<i>Linolenic acid</i>	Fatty acids	10.26
7.	<i>9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)</i>	Fatty acids	15.90
8.	<i>(7-Methyl-4,6,6a,7,8,10a-hexahydroindolo[4,3-fg]quinolin-9-yl)-methanol</i>	Alkaloids	2.99
9.	<i>1,7-Di(dodec-9-ynyl)-2,2,4,4,6,6-hexamethyl-1,3,5,7-tetraoxa-2,4,6-trisilaheptane</i>	Triterpenoid	6.33
10.	<i>(Z)-β-Farnesene</i>	Flavonoids	0.59
11.	<i>Methyl 2-hydroxy-4-methoxybenzoate, trimethylsilyl ether</i>	Hydroquinone	2.04
12.	<i>Tetradecane, 2,6,10-trimethyl-</i>	Alkane	1.03
13.	<i>1-Heptatriacotanol</i>	Alcoholic compound	1.47

3.2. Insecticidal effect

Insecticidal effect from waste extract of two local spices plant on the rice weevil was measured based on their feeding deterrent effects. The difference in weight loss of rice was assumed as feed consumption, and it was an indicator of the insecticidal effect. The results of feeding deterrent effects of both extracts are presented in Table 3. All the extracts had inhibitory effects on the feeding activities of rice weevil. Exposure time has a significant effect on rice consumption ($\alpha < 0.05$) because the remaining weight of rice showed a smaller than control, and the smallest in the 3 hours exposed treatment. Between the two medicinal plant extracts tested, *A. cardamomum* had the higher deterrent effect with a 0.46% weight loss average, and *Z. zerumbet* had the lower effect with 0.60% weight loss (t-test; $\alpha < 0.05$).

Table 3. Percentage of the weight loss of rice on each treatment.

Exposure Time (hour)	Weight loss of Rice (%)	
	<i>Z. zerumbet</i> extract	<i>A. cardamomum</i> Extract
0	1.10 ± 0.136^a	1.10 ± 0.136^a
1	0.49 ± 0.082^b	0.31 ± 0.022^b
2	0.43 ± 0.067^c	0.30 ± 0.112^b
3	0.38 ± 0.075^c	0.14 ± 0.065^c
Averages	0.60 ± 0.021^a	0.46 ± 0.021^b

Notes:

- Value is mean \pm SE. According to ANOVA and Tukey's multiple-comparison tests, the means followed by the same letter in the same column are not significantly different.
- The means followed by a different letter in the average row are significantly according to the student t-test ($\alpha < 0.05$).

In antifeedant tests, extracted oils of *Z. zerumbet* were able to alleviate the consumption of flour disks, especially terpinen-4-ol, because it inhibits feeding behavior in insects [18]. Many phytochemical compounds from plants have been shown to have a disruptive effect on insect organs' performance and, in particular, research on insect grains [19]. Previous studies have been proven that the use of plant powders, oils, and extracts as protectants against rice weevil *Sitophilus oryzae* L. The feeding activity inhibition in rice weevil occurred due to plant chemical treatment [20]. Active plant compounds can disrupt the reproductive phase and prevent female insects from laying many eggs or poisoning females before laying eggs. [21]. There is some likelihood of changes in behavior in the adult insect. Plant volatile oils' biological activity depends on their chemical composition, the variety of extracted plant parts, extraction method, soil properties, environmental conditions, plant age, and harvest method. [22].

According to Singh et al. [23]. Fumigation is one of the best ways to eradicate current storage grain pests. This action is the most effective, practical, and fast and is considered safer because it does not come into direct contact. In this study, adult rice weevil exposed by the extract of the post-harvest waste of *Z. zerumbet* and *A. cardamomum* indicates a change in feeding behavior. After they have inhaled the active compounds in the extracts, there is a decrease in the feeding appetite. Feeding was reduced in rice weevil by extract with various secondary compounds inside it. Similar results have been observed in the ginger family (Zingiberaceae) case to adult *Sitophilus zeamais* and *Tribolium castaneum* [24]. It is known that the active compound of the extract can provoke feeding deterrence in adult rice weevil—Koul [25] of deterrent receptors with broad chemical sensitivity. Finally, The results of this study are expected to be the basis for making bioinsecticide formulations that are inexpensive and environmentally friendly.

4. Conclusion

In conclusion, the waste extract of both plants was able to decrease the feeding activity on the rice weevil. Still, waste extract of *A. cardamomum* is more effective than *Z. zerumbet* extract. These studies demonstrate that waste extract of *A. cardamomum* could be a potent feeding deterrence for rice weevil when an appropriate formulation and application method is developed. The use of plant extracts and their components as bioinsecticides for stored seed insects is sustainable because it is environmentally friendly, inexpensive, and biodegradable.

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