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P Widiyaningrum*, D Candrawati, D R Indriyanti and B Priyono

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang, Indonesia

*Corresponding author: wiwiedeka@mail.unnes.ac.id

Abstract. Post-harvest waste of local cardamom plants (*Amomum cardamomum* Wild.) such as stem and leaves have no economic value but potential as a bioinsecticide. It was known to contain insecticidally active ingredients. This study aimed to analyze the behavioral response of adult *Sitophilus oryzae*, L. under two shapes extract frompost-harvest wasteof local cardamom as the repellent control. Four doses, ranging from 25% to 100% of each treatment were tested using Y- tube olfactometer.The behavioral responsewas observed by the PreferenceIndex(PI) value and Percentage of Repellency (PR). The PI value was analyzed descriptively, while PR was analyzed by two way ANOVA. The data show that PI value is negativeat 75 and 100% doses both of cardamom plant.PR values were significantly under difference of doses and shape,whilethe optimally doses at 100% (LSD; $\alpha<0.05$).PRvalue of extract was 57.6%, higher than the powder which was only 52.8%. It is found that rice weevil adult has the ability to respond behaviorally to post-harvest waste of local cardamom plants, but extract shape is more effective than powder. This study provides positive information about the potential that post-harvest waste of local cardamom as an economically-value bioinsecticide.

1. Introduction
Insect pests are the major problem in storage products, including the Rice weevil (*Sitophylus oryzae* L).Its ability to adapt and breed rapidly causes rice weevil to be difficult to control. According to Antika et al.[1], the attack of rice weevil decreases rice quantity and quality. The quantitative damages could be as weight depreciation, while qualitative damage includes rice being perforated, breakable, discolored, contaminated with dirt, unpleasant odor and decreased nutrient content. Fumigation with chemical pesticides is the most common method used to control product-stored pests, including rice weevil. But for the long term, the use of chemical pesticides must be abandoned because it is feared to cause resistance to insects, environmental pollution and negative effects on non-target organisms and human health [2].

To reach the Good Agriculture Practice, the government through SNI 6729/2016 [3], has issued regulations on organic farming systems, which in principle prohibit the use of chemical insecticides in pest control, and recommend the use of natural pesticides (biopesticides) and mechanical control. Therefore, developing bioinsecticide from natural materials with no economic value today is a strategic alternative. Inexpensive materials, abundant availability and their use do not compete with human needs, are some considerations to produce economically profitable products.

Potential bioinsecticide sources that need to be developed are materials derived from post-harvest wastage of Zingiberaceae plant, one of which is the local cardamom stem and leaves (*Amomum cardamomum* Wild). This Indonesian native plants have not been widely cultivated and used the fruits only as herbs and spices. Approximately 2400 plant families in Indonesia have been identified for their chemical components and 10 thousand of them contain secondary metabolites as potential raw materials for biopesticides, including the Zingiberaceae familia[4,5].In order to the insect pest management, most of the secondary metabolites such as terpenoids and alkaloids are nominee for insecticidal compounds that could be an potential alternative. The mixture of secondary metabolites may be a deterrent to insects and herbivores for the longer period than single compound.

Plant compounds such as fatty acids, essential oils, glycosides, flavonoids, and alkaloids hold anti-insect effects, and their importance as an reserve to the chemical compounds used in the control of insect pest management in different ways.Various studies have proven that secondary metabolites of different types of plants can control insect attacks, through repellent (refuse); antifeedant (preventing / inhibiting appetite); toxicant; growth inhibitor (growth inhibitor); chemosterilant (resulting in sterile / sterile); and attractant (attraction) [6].However, many of the compounds exhibit certain useful biological activities for non-target species. These can be classify into three major chemical classes: alkaloids, phenolics, and terpenoids.Plants that can control insects generally have a characteristic bitter taste (containing alkaloids and terpenes), smells typical and tastes somewhat spicy. Plants with these properties are rarely attacked by pests and are now widely used as botanical pesticides in organic farming [7].Insecticides of plant origin are obtain increased consideration and interest among those concerned with eco friendly, secure and integrated crop management approaches. Furthermore, they are present as vital part in organic food production globally. [8]. Leaves and stems of Zingiberaceae generally have a distinctive aroma similar to rhizomes or fruit, because they contain essential oils [9]. The previous studies found that cardamom leaves contain essential oils ranging from 2.43% [10] and 3.15% [11]. This study was conducted to analyze the behavioral response of rice weevil adult to repellent compounds from post-harvest wastage of local cardamom.

2. Methods

The study was conducted at the Biology Laboratory of Universitas Negeri Semarang, from January to June 2018. Stems and leaves of local cardamom plants were from the Temu Kencana herbal medicine gardens, Sumur Jurang Village, Gunungpati, Semarang. The powders and extracts preparations, active metabolites of secondary metabolites analysis and bioassay tests were done at the Laboratory of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang.

2.1 Powder and extract preparation

Material samples from post-harvest wastage of local cardamom (stem and leaves) in a ratio of 1: 1 were washed, cut into pieces and dried in the shade. Dried samples were milled mechanically and stored in an airtight container. This powder was partially packed in a tea bag for powder treatment tests. Others were macerated in 96% ethanol with a ratio of 1: 1 to obtain 100% extract, and then extract 100% was stored in a dark glass bottle.Some extracts were taken to be evaporated and analyzed its contents of the active compounds using the GC-MS (Gas Chromatography-Mass Spectroscopy) method.

2.2 Rice Weevil Culture

Male and female adult rice weevil were collected from traditional markets in Semarang, then cultured in rice media at room temperature of $28^oC \pm 1^oC$ and humidity of $75\% \pm 5\%$, with a female-male ratio of 1: 3. Adult rice weevil from the first generation (F1) of breeding, unsexing were used for the experiments.

2.3 Behavioral response of Rice Weevil to Repellent Effect

The behavioral response of rice weevil adults to odor stimuli (powder and extract of cardamom plant wastage) was measured by using the Y-shape olfactometertube, similar to those described by Tyagi et al.[12].Olfactometer with 3 connected glass tubes (Y shapes, 10 cm long, 2 cm diameter). Each of tubes were connected with a glass bottle (3.5 cm long, 2 cm diameter), and another end of the tube was used to introduce insects. To confirm the repellent effect,the rice weevils were exposed to 25,50,75 and 100% of powder and crude extract from cardamom plant respectively. Liquid maceration 100% were assumed to be doses of 100% crude extract. To get a dose of 75%, 50% and 25%, liquid extract diluted using 70% alcohol. Powder treatment was also made in 4 levels of treatment, In this study, 100 mg of powder was assumed to be equivalent to 100% (100μL) of crude extract, because it was obtained from the maceration of the material in a ratio of 100 g of powder: 100 ml of 96% ethanol solvent (1: 1). Each treatment was given as much as 100μL of extract, dropped into Whatman filter paper (2cm in diameter). The filter paper was allowed to evaporate for 1 minute, then folded and placed inside the glass bottle, which was attached to each end of the olfactometer's arm. Arm A was given filter paper according to treatment; arm B was only given filter paper without crude extract as a control.

Y-olfactometer was placed horizontally on a white paper. A total of 25 unsexed rice weevil were put in the tube. A total of 25 unsexed rice weevil (test insects) were introduced into the olfactometer. If rice weevil did not like the odor stimuli of powder or crude extract on arm A, they were assumed to be reversing towards the arm without treatment (B). After 60 minutes no displacement occurred, the number of rice weevil found in each olfactometer's arm was recorded. The powder and crude extract of cardamom plant were tested separately in four doses, and each dose was replicated 5 times.Behavioral responses of the repetition of rice weevil due to the effect of treatment was stated as the Preference Index (PI) and Percentage of Repellence (PR). Negative values of PI indicated that post-harvest waste of local cardamom has a repelling effect on rice weevil. PRvalue was used to find out how strong the repellent effect of the material was on test insects. PI and PR were calculated by the following formula [13].

$$PI = \frac{Rt - Rc}{Rt + Rc} PR = [1 - (\frac{Rt}{Rt + Rc})] \, x \, 100\%$$

PI : Preference Index
PR: Percentage repellency
Rt : number of rice weevil trapped in the crude extract or powder test chamber
Rc : number of rice weevil trapped in the control test chamber

2.4 Data analysis

The PI values are presented in graphs and analyzed descriptively. The PR is presented in the table of PR ± SD averages and analyzed the variance of ANOVA and further LSD test ($\alpha < 0.05$), while shape of extract was determined using student t-test.PR data also can be categorized into five PR scale criteria to find out the strength of repellent effect from post-harvest wastage of cardamom plant, as follows [14].

PR > 80%	= very high effect
60% < PR ≤ 80%	= high effect
40% < PR ≤ 60%	= medium effect
20% < PR ≤ 40%	= weak effect
PR ≤ 20%	= very weak effect (there is no effect)

3. Results and Discussion

The stem and leaves of cardamom plants have a similar aroma to the part of the seed. This is a marker there are the same essensial oil compounds in both organs. The results of previous studies revealed that cardamom seeds contain at least essential oil components including α-pinene, ß-pinene, psimene, 1,8-cineol and α-terpineol. The phytochemical compounds in Local Cardamom Stem and Leaf extract by GC-MS showed that cardamom extract was dominated by a high percentage of fatty acid, namely palmitic acid, 13-trans-13-octadecanoic acid and Oleic acid. However, it was also detected essential oils such as p-Cymol; 1.8 Cineole; Sabinol; Isothymol; ascaridole and Piperitone, as well as saponin and flavonoid (Table 1).

Table 1. Phytochemical compounds in local cardamom stem and leaf extract

No	Retention Time (RT)	% Area	BM (g/mol)	Phytochemical Compound	Group of compound
1.	2.604	0.9639	134	*p-Cymol*	Essential oil
2	2.709	0,9635	152	*Sabinol*	Essential oil
3.	2.789	1.0593	154	*Eucalyptol /1,8 Cineole*	Essential oil
4.	4.584	0,5495	150	*Isothymol*	Essential oil
5.	5.915	0,7907	184	*ascaridole*	Essential oil
6.	6.055	1,9262	168	*Piperitone*	Essential oil
7.	10.287	1.1982	302	*Icosapent*	Essential oil
8.	7.075	15.2922	400	*Cholestan 3-ol*	Saponin
9	8.086	1,6536	204	*Valencen*	Flavonoid
10.	10.137	0,9777	222	*Globulol*	Flavonoid
11.	13.438	34.7193	256	*Palmitic acid*	Fatty acid
12.	15.729	30.5096	282	*13-trans-13-octadecanoic acid*	Fatty acid
13.	15.974	15.2922	282	*Oleic acid*	Fatty acid

Based on these results, the waste of cardamom plants has the potential as an economical source of bioinsecticides. Previous studies have proven that secondary metabolites naturally could be as a plant defense against insect pests so that they have the potential as bioinsecticides [15,16].

Production of repellents derived from plants may be easier and cheap than the synthesis of some more complex attractive semio chemicals [17]. Therefore, developing bioinsecticide from natural materials with no economic value today is a strategic alternative. Inexpensive materials, abundant availability and their use do not compete with human needs, are some considerations to produce economically profitable products.

The repellents are desirable chemicals as they offer protection with minimal impact on an ecosystem, and repel the insect-pests from the treated materials by stimulating olfactory or other receptors of insects. Repellents from plant origins are considered safe in pest control operations as they minimize pesticide residues; ensure the safety of the people, food, environment, and wildlife [18]. The plant extracts, powders and essential oils from different bioactive plants were reported as repellents against different economically important stored product insects [19].

3.1 Preference Index value

The response of rice weevil to the reproductive power of cardamom plants can also be seen from the value of the repellency index stated as the Preference Index (PI). The negative PI value indicates the repellent effect of the tested material (Figure 1).

Figure 2 shows the PI value is getting smaller in line with the increasing dose of powder or cardamom plant crude extract. PI expresses polarity of orientation choice, and generally, repellency go up with an increase in treatment doses. The PI ranged from 0.57 to -0.15. Repellent index of the test material showed negative numbers on 75 and 100% on crude extract, while the powder at 100% only. This repellent effect is thought to have originated from essensial oil especially 1.8 Cineol compounds which were detected in post-harvest waste of cardamom plants (Figure 1). The 1.8 Cineol compounds are one of the aromatic compounds in essential oils which have the characteristics of fresh, camphor aroma and spicy flavor. Essential oils are assured to disturb with basic metabolic, biochemical, physiological and behavioral functions of insects pest[20]. Fumigation research showed that the natural oils had a 'knock down effect' on the test insect. Natural oils act by precluding insect acetylcholinesterase (AChE) and thus, eventually blockade the nerve functions [21].

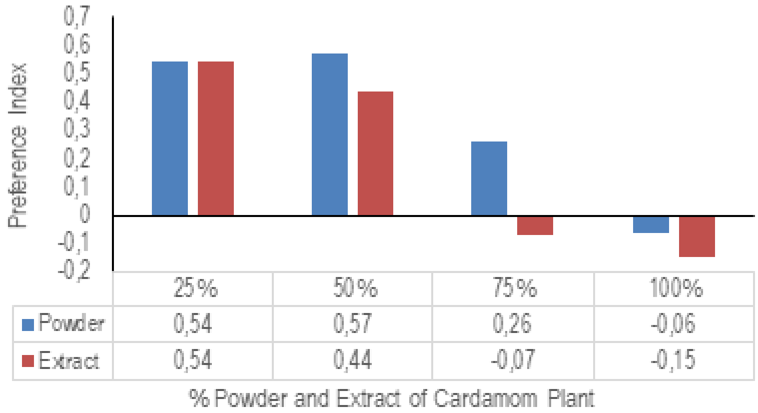

Figure 1. PI values at various doses frompost-harvest waste of local cardamom

Most insects respire by means of the spiracle and trachea. These spiracles might have been inhibit there by distinguished to suffocation [22]. The natural oils obstacle the spiracles, resulting in retardment of the respiratory system [23]. Various natural fatty acids have insecticidal trait [24].

3.2 Percentage of repellence

The response of rice weevil to the repellent effect of powder and extract cardamom plant can be seen from the percentage of repellency (PR). Table 2 shows that various doses and extract shapes significantly affected the PR value. Likewise, the interaction of both treatments also had a significant. Furthermore, LSD-test showedthat PR increases with an increase in treatment doses.The highest PR value is found in 100% doses, both in powder and crude extracts. The student t-test also founding the post-harvest wastage of crude extractwas significantly higher ($\alpha < 0.05$) than PR value at powder treatment (Table 3).

Table 2. Variance analysis of treatments

Source	df	Mean Square	F	Sig.
Various Doses	3	2459.067	126.756	.000
Extract shapes	1	490.000	25.258	.000
Doses * Extract	3	125.200	25.258	.002

Table 3. Percentage of repellency of cardamom powder and extract to rice weevil

Powder (mg)	PR (%)	Extract (%)	PR (%)
25	23.2 ± 5.9329^{a}	25	23.2 ± 5.2153^{a}
50	21.6 ± 4.5607^{a}	50	28.0 ± 2.8284^{a}
75	36.8 ± 3.3466^{b}	75	53.6 ± 4.5607^{b}
100	52.8 ± 3.3466^{c}	100	57.6 ± 4.5607^{c}
Average	33.6 ± 13.5117^{a}		40.6 ± 16.0669^{b}

Notes: Indexes a,b in the rows of average indicate significant differences (Student t-test; α<0. 05); superscript letters in both columns indicate statistically significant (LSD; α< 0.05)

In high concentration, the natural oil from cardamom acts as a potential grain protectant by thuggery various life stages of the insects attacking wheat, such as T.castaneum and S. zeamais, via contact and fumigant action. In this study, the post-harvest wastage from cardamom stems and leaves is known to contain oil essential as like in seeds, but the concentration is low. Therefore, the bioassay-test on rice weevil causes repellent effects only doesn't have an impact on mortality. According to Abbasipour et al [25]the essential oil of *Elettaria. cardamomum* toxic to the flour moth (*Ephestia kuehniella* Zeller), the red flour beetle (*Tribolium castaneum* Herbst) and the bruchid beetle (*Callosobruchus maculatus* Fabricius).Ebadollahi & Sendi [26] proved that *Elettaria cardamomum* have repellent activity against *Sitophilus zeamais*.

Based on the established criteria, the use of powder up to 100 mg gives a medium category repellent effect (PR 52.8%). But in the extract treatment, the repellent effect was seen at 75% and 100% extract dosages with a range of 53.6 - 57.6%. It indicates that cardamom waste in extract form gives a better repellent effect than cardamom waste in powder. It means rice weevil had given a rejection response to cardamom extract at a dose of 75%, while the rejection response to new powder is detected by rice weevil at a dose of 100 mg. It happened because the aromatic compounds in the extract evaporated faster and spread more widely than aromatic compounds bounded in the powder.

The medium repellent effect is thought to be due to the low content of the active compound, while the ratio of liquid extract to the unit area of the Y-olfactometer tube used is thought to be too little. The volume of the Y-olfactometer tube is approximately [3.14 x (2.3) 2] x 10 cm^3 = 180 cm^3 (= 180,000 μL). Liska et al. [27] found that the use of high-dose of 1.8 Cineol (100μL / 720 mL) was able to repel the red beetle (*Tribolium castaneum* Herbst.)) with the success of 92.5%.Based on the results of this study,is to provide positive information about the potential that post-harvest wastage of local cardamom as an economically-value bioinsecticide.

4. Conclusion

It is found that rice weevil adult has the ability to respond behaviorally to post-harvest waste of local cardamom plants, but extract shape is more effective than powder. This study provides positive information about the potential that post-harvest waste of local cardamom as an economically-value bioinsecticide. The findings of this study also inform farmers that bioinsecticide can be easily prepared using the simple and available materials.

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