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Research Article
Antifeedant Effect of Some Medicinal Plant Extracts Against Rice Weevil

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Abstract

Background and Objective: At present, insecticides with raw materials of active compounds from plants have become a strategic alternative, because they are easily obtained and produced. In an attempt to find natural and cheaper methods for the control of rice weevil (*Sitophilus oryzae* L.), some useless waste of local medicinal plant extracts was evaluated. This study aimed to evaluate the antifeedant effect of various medicinal plant extracts on rice weevil (*Sitophilus oryzae* L.). **Materials and Methods:** The experimental study using a completely randomized design of two factors, i.e., source of the extract in 7 types of medicinal plant waste and the exposure time of extracts in 0, 1, 2 and 3 h. The antifeedant effects were evaluated after 3 weeks by measuring feed consumption (FC), feeding deterrent index (FDI) and rice weevils' mortality. Data were analyzed using variance analysis. **Results:** The statistical analysis showed that the differences in exposure time contributed significant influence ($\alpha \leq 0.05$) on FC, FDI and mortality. The lowest FC occurred at the 3 h exposure treatment, i.e. on the extract of *Amomum cardamom* plant, while the highest FC was found in the treatment of *Curcuma mangga* plant extract. The values of FDI and mortality at the 3 h extract exposure were 31.53-87.16% and 42.4-64%, respectively with the highest value in the treatment of *A. cardamomum.* **Conclusion:** At last, these findings inform peoples that the waste of medicinal plants, especially cardamom is possible to be developed as botanical insecticides for rice weevils.

Key words: Antifeedant, extracts, *Amomum cardamom*, feed consumption, feeding deterrent index, medicinal plants waste, rice weevil

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The common pest found in stored products such as grains, rice and flour belongs to the family of Coleoptera. One of which is rice weevil (*Sitophilus oryzae*). Besides physically damaging the stored rice and grains, this species is responsible for the entrance of pathogenic organisms, such as fungi or bacteria[1,2]. Further, the rice weevils which have infected the stored rice are difficult to control because of their fast breeding. According to Manueke *et al.*[3] an adult female rice weevil can lay 300-400 eggs in one life cycle with an average sex ratio of 0.79.

A preventive action to control pests in intervention stores is generally done by using synthetic insecticides such as phosphine and methyl bromide through fumigation. Even though a synthetic fumigant is fast in cleaning up pests, ecologically, it has adverse effects since it wipes out non-target organisms and leaves poisonous residue for humans and environment as well as causes insect resistance if used continuously[4].

Currently, botanical insecticides have been an environment-friendly alternative for controlling pests. They are relatively safe for humans and environment, cause no resistance, are compatible with various control methods and produce healthy agricultural products since they are free from synthetic insecticides residue[5,6]. Natural insecticides are considered more prospective to be developed because they have abundant raw material sources and have simple manufacturing technology, so farmers will quickly adopt them[7]. Natural or botanical insecticides generally do not directly kill target pests but work by inhibiting growth, development and reproduction of insects (insect growth regulator), or changing insect behavior in the form of pheromones, repellent, attractant and antifeedant[8].

An antifeedant or feeding deterrent is described as a substance that causes a decrease in insects' eating activity. This characteristic is found in almost all plant secondary metabolites such as essential oils, alkaloids, phenols, terpenoids flavonoids, glycosides, esters and fatty acids. These materials also anti-insect compounds and have potential as botanical insecticides[9].

A group of plants reported having insecticidal activities are plants from Zingiberaceae family. Studies on secondary metabolite from plant rhizomes reveal that the members of Zingiberaceae which have the potential as a source of natural insecticides are turmeric rhizome (*Curcuma longa*)[10], ginger (*Officinale zingiber*)[11], cardamom (*Amomum cardamomum*)[12], galangal (*Alpinia galanga*)[13] and bitter ginger (*Zingiber zerumbet*)[14]. However, the rhizome of medicinal plants generally has high selling value as raw materials for spices and herbal medicines, so they are less economical if used as a source of botanical insecticides. By referring to the previous explanation, the purpose of this study was to analyze the content of the active compounds of postharvest waste of seven plants belonging to Zingiberaceae and to test the effect of antifeedant extracts on the rice weevil eating activities.

MATERIALS AND METHODS

Extracts preparation: The study was carried out at Universitas Negeri Semarang, Biology Lab from February-August 2018. Local herbal wastes (stem and leaf) used in the antifeedant test (Table 1) were obtained from Temu Kencono herbal garden in Sumur Jurang village, Gunungpati- Semarang. The mixture of stems and leaves (ratio 1:1) of each sample was washed and dried at room temperature $\leq 50°C$ and then it was ground into powder. About 250 g of powder was macerated using ethanol solvent with a ratio of 100% (g/v) for 24 h and then squeezed and filtered using Whatman filter paper to separate the extract from the residue. The collected extract was concentrated using a rotary evaporator to obtain the concentrated extract. This extract sample was phytochemicals analyzed using the GC-MS method (Gas Chromatography-Mass Spectrometry), while others were used for the antifeedant test. This concentrated extract assumed to extract 100% concentration.

Insect preparation: Adult male and female rice weevils obtained from the traditional market were bred in 20 jars. Each was filled with 50 g of rice, 20 adult male and female rice weevils (ratio 1:3) and then tightly closed using gauze (muslin cloth). The breeding of th e rice weevils was conducted in a

Table 1: Medical plants used in antifeedant tests

Local name	Botanical name	Family name	Parts used
Kapulaga	*Amomum cardamomum* Willd.	Zingiberaceae	Stem and leaf
Lempuyang	*Zingiber zerumbet*	Zingiberaceae	Stem and leaf
Kunyit kuning	*Curcuma longa*	Zingiberaceae	Stem and leaf
Kunyit putih	*Curcuma mangga*	Zingiberaceae	Stem and leaf
Temu kunci	*Boesenbergiae rhizoma*	Zingiberaceae	Stem and leaf
Temu lawak	*Curcuma xanthorrhiza*	Zingiberaceae	Stem and leaf
Lengkuas	*Alpinia galanga*	Zingiberaceae	Stem and leaf

room temperature of 27±2°C, humidity of 75±5%. Every week, the rice weevils were moved to other breeding jars, while the containers and the eggs left were incubated until imago (adult rice weevil) appeared. The imago from the results of this hatchery was assumed to be the first offspring (F1 generation) and they will be used as a test insect.

Evaluation of the antifeedant effect: Seven types of local medicinal plant waste extracts were analyzed for their active compounds and tested to rice weevils to reveal whether the waste extracts showed an antifeedant effect. In this phase, the researchers conducted an observation using a completely randomized design (CRD) of two factors. The first factor was the source of the extract consisting of 7 types of herbal plant waste (Table 1), the second factor was the exposure time of extracts (0, 1, 2 and 3 h). Additionally, the antifeedant effect was measured by calculating the Feeding Deterrent Index (FDI) in percent, feed consumption (%) and mortality.

A total of 100 μL of concentrated extract from each plant waste was dripped onto filter paper (2.5 cm in diameter) placed in a petri dish (10 cm diameter). Then, it was aerated for 30 sec. A total of 25 adult rice weevils were put into a petri dish and then closed to make them standstill. The duration of exposure to extracts was given according to the treatment of 0, 1, 2 and 3 h. Rice infestation that has been exposed to the extract was transferred to a plastic jar measuring 3.5×3.5×7 cm containing 10 g of rice. The plastic jars were covered with gauze. All treatment jars were incubated in a dark room with a room temperature of 28±1°C and humidity of 75±5%. For the control treatment, a jar containing 10 g of rice was filled with 25 insects without exposure to the extract. Each treatment combination was repeated five times. Meanwhile, the rice lice were kept for 3 weeks. Each week, the jar was opened to calculate the shrinkage of rice and the number of dead rice weevils. Moreover, the average feed consumption in percent (K) was calculated using the Liu's formula[15]

$$K\ (\%) = \left[\frac{(K1 + K2 + K3) \div 3}{25}\right] \times 100$$

While, FDI was calculated using as follows:

$$FDI\ (\%) = \frac{C - T}{C} \times 100$$

where, K1, K2, K3 were feed consumption in weeks 1, 2 dan 3. C is the average feed consumption in the control group, T = feed consumption in the treatment group.

Statistical analysis: Data on the percentage of feed consumption and mortality were analyzed using variance analysis (ANOVA). If the results of the analysis showed a significant effect ($\alpha \leq 0.05$), they would be followed by a different LSD test to find out which treatment contributed to the maximum antifeedant effect. The FDI value (%) was analyzed descriptively using the FDI criteria according to Liu *et al.*[15] as follows:

- FDI<30% : Any antifeedant effects (-)
- 50%>FDI≥30%: Antifeedant effects are weak (+)
- 70%>FDI≥50%: Antifeedant effects are medium (++)
- FDI≥70%: Antifeedant effects are strong (+++)

RESULTS AND DISCUSSION

Phytochemical compound of extracts: The antifeedant describes as a substance that can reduce the ability to eat insects. This compound is found as a main secondary metabolite, namely alkaloid, phenolics and terpenoids compounds, including essential oils. Besides, the active compounds of plants consisting of essential oils, flavonoids, alkaloids, glycosides, esters and fatty acids have anti-insect effects and are potential as a source of bio-insecticides[16]. Table 2 showed the result of the GC-MS analysis of seven extracts of medicinal plant wastes, tabulated into five phytochemical compounds potential as an antifeedant.

In this study, the seven plant extracts were known to contain 5 classes of active compounds in various quantities. All extract samples contained fatty acids. In detail, terpene

Table 2: Result of the GC-MS analysis of seven extracts of medicinal plant wastes

Sources of extract	Phenols	Terpenoids and essential oils	Flavonoids	Alkaloids	Fatty acids
A. cardamomum	1	7	2	1	6
Z. zerumbet	-	1	1	1	6
C. longa	-	6	2	1	7
C. mangga	1	3	-	-	7
B. rhizome	1	1	4	-	4
C. xanthorrhiza	1	-	-	-	4
A. galanga	1	6	1	1	4

Number indicates the number of compounds detected compounds and essential oils were detected in 6 extract samples, except *C. xanthorrhiza* plant extract. Alternatively, the most essential oil compounds were found in *A. cardamomum, C. longa* and *A. galanga* plant extracts. Whereas, flavonoid groups, phenols and alkaloids were also detected, but not in all samples. Hence, the extract which contained 5 groups of active compounds was the extract of *A. cardamomum* and *A. galanga* plants.

Feeding deterrent index (FDI): According to the results of FDI, the rice weevils which were exposed to the extracts for 3 h experienced various antifeedant effects ranging from 31.53 -87.16% and the results of the mean statistical analysis of FDI revealed significant differences between the treatments (LSD<0.05). The highest value on FDI was found in the treatment using *A. cardamomum* plant extract, while the lowest was in *C. xanthorrhiza*. This shows that different extract sources are affecting FDI (Fig. 1).

Apparently, the exposure time and types of extract also contributed significant effects to feed consumption (Table 3). The results of the LSD test ($\alpha \leq 0.05$) showed that the treatment of 3 h exposure resulted in maximum feed consumption, FDI and mortality rates compared to one and two hours exposure. The feed consumption of the rice weevils

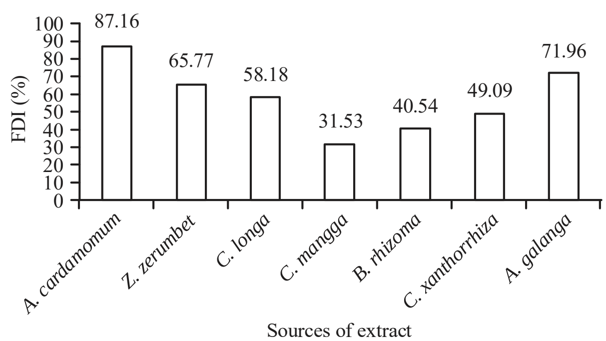

Fig. 1: Percentage of FDI in various medicinal plants extracts with a 3 h of exposure

exposed to *A. cardamomum* plant extract for 3 h gained the lowest number compared to other treatments. Meanwhile, the highest mortality of the rice weevils at the end of the 3rd week was found in the treatment of the extracts of *A. cardamomum* and *A galanga*. This indicated that all types of extract produced antifeedant effects in varying percentages.

If confirmed by qualitative criteria, the strong antifeedant effect (FDI>70%) in this study was only found in the extracts of *A. cardamomum* and *A. galanga.* Meanwhile, *Z. zerumbet* and *C. longa* produced the medium effect, while *C. manga, B. rhizome* and *C. xanthorrhiza* had a weak effect. The extracts of *A. cardamomum* and *A. galanga* were found having five active anti-insecticides components, including 7 and 6 essential oil compounds. These plant extract samples also contained phenol, alkaloids, flavonoids and fatty acids compounds. Whereas the researchers found that the *C. longa* extract contained compounds of essential oils but no phenol compound. The absence of this compound was assumed as the reason why this plant extract had a lower antifeedant effect than *A. cardamomum* and *A. galanga.* For more, the number of other active compounds detected other than essential oils were assumed to jointly cause a stronger/ stinging aroma for insects, so that the extracts of A. cardamomum and *A. galanga* in 3 h exposure were stronger to affect the performance of taste organs and affect eating activities. As a result, less feed consumption occurred. This condition will result in a higher FDI. According to Syahputra[17], the antifeedant effect arises because of the presence of substances (active compounds) that are inhaled or touched taste organs, disrupting signals that stimulate appetite and then affecting eating activities. The stronger the aroma of the compounds absorbed by the insect's body, the stronger the ability to eat and ultimately the consumption of feed decreases. Again, Wicaksono *et al.*[18] state that the antifeedant effect can obstruct the ability to eat, even stops eating activity in insects due to disorders of peripheral sensilla

Table 3: Average feed consumption by rice weevil during three weeks of treatment (%)

	Exposure time				
Sources of extract	0	1	2	3	Average
A. cardamomum	1.09±0.146	0.31±0.022	0.30±0.112	0.14±0.065	0.46±0.086[a]
Z. zerumbet	1.11±0.131	0.49±0.082	0.43±0.067	0.38±0.075	0.60±0.089[b]
C. longa	1.10±0.136	0.86±0.083	0.80±0.122	0.46±0.054	0.80±0.099[c]
C. mangga	1.11±0.131	0.98±0.148	0.98±0.178	0.7±0.089	0.96±0.136[d]
B. rhizoma	1.11±0.131	0.92±0.114	0.76±0.114	0.66±0.148	0.86±0.127[c]
C. xanthorrhiza	1.10±0.136	1.10±0.122	0.98±0.148	0.56±0.114	0.93±0.130[d]
A. galanga	1.07±0.149	0.74±0.089	0.66±0.054	0.30±0.100	0.69±0.098[b]
Average	1.10±0.137[a]	0.77±0.094[b]	0.70±0.114[b]	0.46±0.092[d]	

Difference of letters on the same row/column shows differences (LSD test, $\alpha \leq 0.05$)

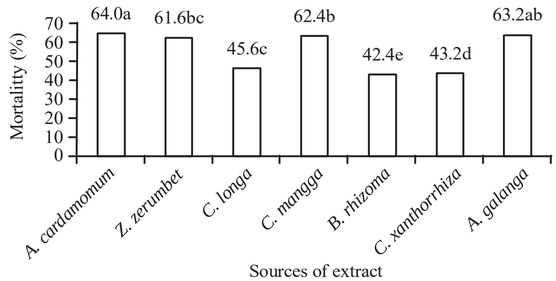

Fig. 2: Mortality of rice weevil in the 3rd week with 3 h of exposure
Difference of letters shows different significant values (LSD test, α ≤0.05)

(taste organs). Additionally, Mastuti[19] states that phenol, (taste organs). Additionally, Mastuti[19] states that phenol, flavonoids, alkaloids, terpenes and essential oils compounds from plants naturally act as self-defense tools against herbivores, including pests.

Rice weevils' mortality: Under the observation of rice weevils' mortality (Fig. 2), it was known that the highest mortality took place in the treatment of *A. cardamomum* plant extract, which based on different LSD tests (α<0.05) had no significant differences from the treatment using *A. galanga* extract. The high mortality was due to the amount of the content of secondary metabolites, especially the components of essential oils which actively acted as a food inhibitor more than other extracts. These active compounds directly blocked the work of sensory cells and within three weeks and caused insects to die of starvation. Meanwhile, antifeedant worked by stimulating specific eating repellent nerves in the form of chemoreceptors found in the mouthpart. These chemical receptors work together with other chemical receptors and cause a disturbance in the perception of stimulation to eat[20,21].

The results of this study are strengthened by Suthisut *et al.*[22] which used essential oils of the 3-plant rhizomes of Zingiberaceae (*Alpinia conchigera* Griff, *Zingiber zerumbet* Smitt and *Curcuma zedoaria*). The results of their study show that the essential oils of the three plants influenced the eating activity of the adult rice beetle. Also, Koul[23] argues that the antifeedant effect does not always occur after food is ingested, but it can also be due to the sensitivity of insect receptors when exposed to volatile compounds appearing from the extract. According to Lina *et al.*[24], active compounds in higher concentrations can be toxic so that when entering into the digestive tract, they will inhibit the performance of digestive enzymes. Moreover, essential oils are one of the secondary metabolites of plants that generally contain a mixture of volatile monoterpenes, sesquiterpenes and phenols that play an important role in defense against insects, some of which have a role as contact poisons[25]. At last, other metabolite compounds generally have antifeedant and repellent effects[26]. This study discovers the possible synergistic effect of some metabolite compounds in medicinal plant extract, that can be beneficial for biological control of rice weevils.

CONCLUSION

In conclusion, all types of extracts have an antifeedant effect on rice weevils, but the strongest effect was found in the extract of *A. cardamomum* in 3 h of exposure. At last, these findings inform people that the waste of medicinal plants, especially *A. cardamomum* is possible to be developed as botanical insecticides for rice weevils.

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