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BIOACTIVITY OF FRAGRANT PANDAN AND WULUH STARFRUIT COMBINATION LEAF POWDERS AGAINST THE MORTALITY OF RICE WEEVIL (*Sitophilus oryzae*)

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ABSTRACT

Storing rice for a long time in the warehouse has the potential to be attacked by warehouse pests, namely rice weevil insect (*Sitophilus oryzae*). Rice weevil attacked on storage rice caused a decrease in the quality and quantity of rice, so environmentally friendly pest control is needed, such as the used of bioactive plant compounds as botanical insecticides. This research aimed to see the effect of bioactive compounds from the combined powder of fragrant pandan (*Pandanus amaryllifolius*) and wuluh starfruit (*Averrhoa bilimbi*) leaves on the mortality of the rice weevil pest *S. oryzae*. The method of this research used a non-factorial Completely Randomized Design (CRD) experimental method with 4 concentration levels of botanical insecticide combination powder, that were 30, 40, 50 and 60 g. The research results showed that the combination of fragrant pandan and wuluh starfruit leaf powders contained bioactive compounds were steroids, terpenoids, saponins, flavonoids, phenolics and tannins. The activity of the bioactive compounds saponins, flavonoids, phenolics and tannins worked as inhibitors of insect feeding (antifeedant) and caused the mortality of *S. oryzae*. The highest mortality percentage reaching 85% was obtained after applicated by a concentration of 50 g. The increase in mortality of the *S. oryzae* pest occurred every week, until mortality reached more than 80% in the 3rd week was obtained in treatment concentrations of 30 g (84.2%) and 50 g (85%).

Introduction

Rice is one of the main food commodities in the world besides corn and wheat. The ever-increasing need for rice causes the need for appropriate storage efforts to keep the rice supply available. However, in the process of storing rice, obstacles are often encountered were attack by rice weevils (*Sitophilus oryzae*) (Mastuti *et al*., 2020). Rice weevils (*S. oryzae*) are able to eat the entire grain so they are classified as the main pest of post-harvest storage warehouses. The characteristic of rice that was attacked by rice weevil pests would be have irregular holes in the grain due to rice weevil bites

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which cause brittleness of the rice. Loss of weight grain and holes in rice were determined the level of damage to rice due to the pest *S. oryzae* (Juniarti *et al.*, 2022).

Quality controls rice are needed to maintain the quality and quantity of stored rice products. Currently, control of insect pests still relies on the use of synthetic pesticides which cause environmental pollution. Control using fumigants against warehouse pests apart from causing environmental damage, can also caused harm to humans and other living organisms (Bakar *et al.*, 2022). Therefore, more environmentally friendly alternative controls are needed by using plant-based insecticides. The compound of bioactive substances in plants can caused feeding inhibition of the activity of insect larvae, characterized by a slow movement response which the antifeedant effect can obstruct the ability to eat until the larvae stop eating and then died (Analisa *et al.*, 2022).

Several types of plants that contain bioactive compounds have the potential to act as natural insecticides. Plants that can be used as botanical insecticides include fragrant pandan and wuluh starfruit leaves. These two types of plants are very easy to find and are generally used as cooking ingredients. According to Ahmed *et al.* (2018), the bioactive compounds contained in wuluh starfruit (*Averrhoa bilimbi*) leaves are alkaloids, flavonoids, saponins, terpenoids, triterpenes, and phenolics. The secondary metabolite compounds contained in fragrant pandan (*Pandanus amaryllifolius*) leaves are saponins, polyphenols, flavonoids, essential oils and alkaloids (Murdani *et al.*, 2023). Chemical compounds that function actively as insecticides and larvicides are saponins and flavonoids. Saponin compounds are stomach poisons that can inhibit the feeding activity of larvae, while flavonoid compounds can be contact and respiratory poisons so they disrupted the body's metabolic processes (Wahyuni & Sari, 2021). By referring to the previous explanation, this research was conducted to analyze the bioactivity of fragrant pandan (*Pandanus amaryllifolius*) and wuluh starfruit (*Averrhoa bilimbi*) leaf powders against the mortality of the rice weevil pest (*Sitophilus oryzae*).

Materials and Methods

Insect Breeding

The insect used was the rice weevil *Sitophilus oryzae* which was collected from rice storage in Meulaboh, West Aceh. The rearing process was carried out by inserting 100 *S. oryzae* adults into 5 jars for 3 weeks, each jar contained 100 g of rice and 20 *S. oryzae*. Insect breeding tests were carried out at the Protection Laboratory, Faculty of Agriculture, Teuku Umar University.

Preparation of Plant-Based Insecticide Powder

Fresh fragrant pandan and wuluh starfruit leaves are taken directly from the land and washed first. Then the leaves are air-dried for 4 to 5 days. after the leaves are dry, then blended using a grinder until the powder is obtained. The refined leaf powder is ready to be used as botanical insecticide.

Botanical Insecticide Testing

This research used a non-factorial Completely Randomized Design with 4 concentration levels of insecticide powder combinations, namely 30, 40, 50, and 60 g, with a combination ratio of fragrant pandan leaf powder and wuluh starfruit each concentration of 1:1. Each treatment jar was filled with 200 g of rice and added plant powder with each concentration level used, namely 30 g, 40 g, 50 g and 60 g. Shake the jar for mixed the plant powder the rice grains. 40 rice weevil imago were

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added to each treatment jar. As a control, a jar containing only 100 g of rice and 40 rice weevil imago without conducted the botanical insecticide.

The mortality of *Sitophilus oryzae* after application of the combination powder was observed for 7, 14, and 21 DAT. Each dead insect was removed from the jar and the number recorded. According to Suanda & Delly Resiani (2020), the mortality percentage is calculated using the formula:

$$P = \frac{n}{N} \times 100\%$$

Information:
P = Percentage of dead insects
n = Number of dead insects
N = Number of used insects

Data Analysis

The mortality data were analyzed witg Analysis of Variance (ANOVA) using IBM SPSS Statistics 25 Software and further tested to see the significant difference between each treatment with DMRT 5%.

Results and Discussion

Bioactive Compounds of Combination Leaf Powder

The results of phytochemical testing to determine the bioactive compounds contained in the combination powder samples of fragrant pandan and wuluh starfruit leaves showed that the combination powder contained steroid, terpenoids, saponins, flavonoids, phenolics, and tannins compounds (Table 1). The results of Aini & Mardiyaningsih (2016) research also stated that pandan leaves (*Pandanus amaryllifolius* Roxb.) contained secondary metabolites in the form of alkaloids, tannins, flavonoids, saponins, and polyphenols. Bioactive flavonoid, saponnins, and tannins compounds are also found in wuluh starfruit (*Averrhoa bilimbi*) leaves (Putra *et al.*, 2018).

Table 1. Result from phytochemical test of powder combination of fragrant pandan and wuluh starfruit

Bioactive Compounds	Reagent	Inference
Alkaloids	Mayer	-
	Wagner	-
	Dragendorff	-
Steroids	Liebermann-Burchard's test	+
Terpenoids	Liebermann-Burchard's test	+
Saponins	Frothing test	+
Flavonoids	Shinoda's test	+
Phenolic	$FeCl_3$	+
Tannins	Gelatin+H_2SO_4	+

Bioactive substances contained in the plants such as flavonoids, tannins, saponins, and steroids provided a mortality effect on corn cob borer insects (*Helicoverpa armigera* Hubner). The active compounds contained in bintaro leaf flour extract enter the body of *H. armigera* larvae as stomach poisons and contact poisons (Analisa *et al.*, 2022).

Insect death due to the toxic bioactive compounds of phenols, saponins and alkaloids in starfruit leaf extract, is characterized by changes in the body to become darker and smaller. This is caused by irritation of the digestive system and

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obstruction of electron transport in the mitochondria due to the absorption of chemical compounds into the insect's body, so that the cells become inactive and eventually died (Setyawan *et al*., 2021). Research conducted by Hasinu *et al*. (2014) also stated that the bioactive flavonoid compounds contained in papaya leaf extract worked as inhibitors of the respiratory process so that the test insects moved to avoid the treated feed.

Rice Weevil *(Sitophilus oryzae)* Mortality Percentage(%)

Based on the results of quantitative analysis of mortality percentage data using the ANOVA test, the results showed that the application of botanical insecticide powder from a combination of wuluh starfruit and fragrant pandan leaves had an effect on the mortality of rice weevil pests at 7, 14 and 21 days after application. The results of the 5% DMRT test in Table 2 showed that the control treatment without the addition of combination powder on the 21st day of DAT, had the lowest percentage of 24.2% and was significantly different from the concentration treatment of 30g (84.2%), 40g (74.2%), 50g (85%), and 60g (45%). Rice weevil pest mortality should not occur in control treatments. However, the mortality of test pests in control can be influenced by external factors such as environmental factors and the old age of the pests (Haris *et al*., 2023).

Treatment with a concentration of 50g on day 21 showed the highest mortality percentage was 85%. This mortality percentage was not significantly different from the treatment at concentrations of 30g and 40g, but was significantly different from the treatment at a concentration of 60% which only reached 45%. The increase in the concentration of botanical insecticide powder used is not directly proportional to the increase in the percentage of mortality of the rice weevil pest (S. oryzae). This can be caused by the bioactive compounds in the plant material being volatile, causing an increase in the number of S. oryzae imago and reducing rice weevil mortality. According to Khasanah *et al*. (2015), due to the evaporation, oxidation and resinification processes, caused the decresing bioactive components contained in the leaves if it stored at longtime.

Table 2. Mortality percentages of *S. oryzae* after treatment with combination leaf powders in 7, 14, and 21 days after treatment (DAT) (mean±SEM)

Treatments	Mortality of *S. oryzae* (%)		
	7 DAT	14 DAT	21 DAT
Control	5.8 ± 1.4 a	18.3 ± 8.0 a	24.2 ± 11.8 a
30 g	25.8 ± 5.2 b	56.7 ± 14.4 b	84.2 ± 20.9 b
40 g	25.8 ± 11.5 bc	51.7 ± 11.8 bc	74.2 ± 3.8 bc
50 g	27.5 ± 10.9 b	50.0 ± 10.9 b	85.0 ± 18.03 b
60 g	13.3 ± 6.3 c	26.7 ± 8.04 c	45.0 ± 13.2 c

*Means in a column with the same letter are not significantly different ($p \leq 0,05$) according to DMRT'S tests

In addition, the increasing concentration of powder used, the stronger aroma of bioactive would be produced, causing the S. oryzae insect to refuse to consume rice grains so that toxic bioactives do not enter directly into the insect's body. According to Lisdayani & Sari (2020), the aroma produced by plants as a form of their adaptability can act as an attractant or repellent for insects. The secondary metabolite content of phenols in

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plants also works as a repellent for insect feeding (Analisa *et al*., 2022).

Increased mortality of the rice weevil S. oryzae occurred every week for all plant insecticide concentration treatments. Based on Figure 1, it showed that the mortality graph continued to increase with each additional observation time. Treatment of powder concentration of a combination of fragrant pandan leaves and wuluh starfruit against S. oryzae in the 1st week (7 DAT) to the 2nd week (14 DAT) did not reach 80% mortality. The mortality percentage of S. oryzae pests exceeding 80% occurred in the 3rd week of observation (21 DAT) and was obtained in treatment concentrations of 30 g (84.2%) and 50 g (85%). Azwana *et al.* (2019) stated that botanical insecticides were effective if application of plant-based insecticides on test pests resulted in more than 80% mortality.

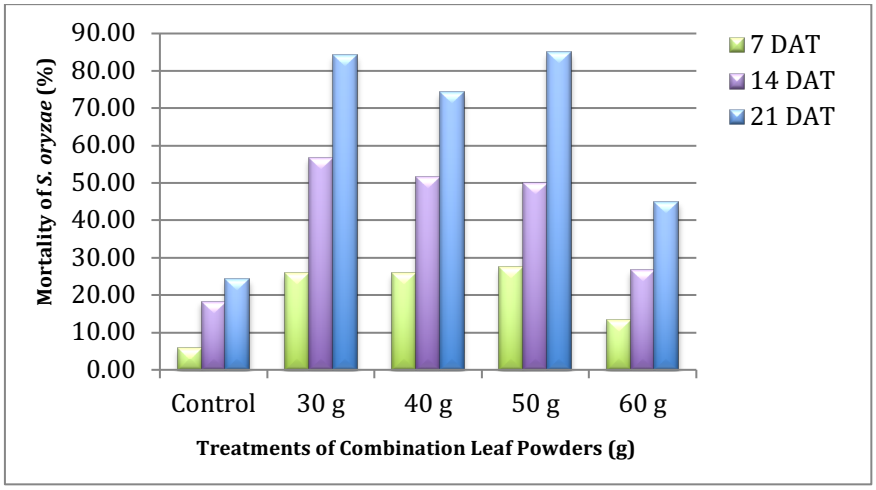

Figure 1. Mean mortality percentages of *S. oryzae* at different days post-treatment (7,14, and 21 days)

Mortality of warehouse pests (*Sitophilus zeamais*) after applicated by kaffir lime leaf extract insecticide increased with increasing observation time. The mortality percentage of *S. zeamais* at a concentration of 15% after 24 hours of application was 10%, and continued to increase to 82.2% after 72 hours of application (Wulansari *et al*., 2019). The augment in mortality which continued to increase until the 21st day in all treatments was thought to be because after treatment, the bioactive compounds contained in botanical insecticides functioned as insect killers (Isnaini *et al*., 2015).

Penetration of bioactive compounds from botanical insecticides into the insect's body can cause nervous disorders due to inhibiting the work of the acetylcholinesterase enzyme, resulting in death. Apart from nervous disorders, plant-based insecticide powders that contain toxic bioactives also disrupted the insect's respiratory system by covering the respiratory organs, resulting in dehydration, leading to death (Yuliani & Jadmiko, 2023). Saponins and tannins substances as toxic can inhibit growth which causes a feeding inhibition mechanism for insects (Asikin & Susanti, 2018).

Conclusions

The powdered combination of fragrant pandan leaves and wuluh starfruit contained bioactive compounds in the form of steroids, terpenoids, saponins, flavonoids, phenolics and tannins. The

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activity of the bioactive compounds saponins, flavonoids, phenolics and tannins worked as inhibitors of insect feeding (antifeedant) and caused the mortality of insects. The highest mortality percentage reaching 85% was obtained after applicated by a concentration of 50 g. The increase in mortality of the *S. oryzae* pest occurred every week, until mortality reached more than 80% in the 3rd week of observation (21 days after treatment). Further testing is needed to see the level of rice damage caused by rice weevils after application of botanical insecticides.

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