EFFECTIVENESS TESTS OF VEGETABLE INSECTICIDES
ESSENTIAL OILS OF MUTMET SEED (Myristica fragrans
Houtt.) AND CLOVE FLOWER (Syzygium aromaticum L.)
ON RICE BEETLE MORTALITY AND DEVELOPMENT

Sitophilus oryzae (Linnaeus, 1763)

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Abstract
This study aims to analyze the inhibition of the development of the F1 generation of rice beetles by nutmeg and clove flower essential oils, and to determine the mortality of rice beetles against nutmeg and clove flower essential oils. The stages of the research started from rearing the rice beetle, preparing test insects, making variations in doses, treatments, and analysis. Two tests were conducted in the study, the first for inhibition of F1 generation activity and the percentage of mortality using the contact method (filter paper). The results showed that nutmeg and clove oil had an effect on the inhibition of the rice beetle F1 generation. Nutmeg oil has the best inhibition power with the percentage of F1 generation inhibition up to 100%. The mortality test using the filter paper method showed that for 48 hours nutmeg essential oil was able to kill rice beetles at all concentrations, namely 5-20% to 100%, while the percentage of 100% mortality was only found in the treatment with clove oil at the highest concentration (20%). The results showed that nutmeg oil showed better results than clove oil for controlling S. oryzae warehouse pests.

Keywords: Cloves, Essential Oils, Nutmeg, Rice Beetles

INTRODUCTION
In Indonesia, rice is the staple food for the majority of the population with a consumption rate in 2016 reaching 98.01 kg/capita/year (Kamsiati, 2018). The need for rice in Indonesia has increased, in line with the increase in population. However, problems arise during storage, namely pests that can reduce the quality of rice. Pest attack on rice during storage (modern and traditional) can cause damage and loss of food weight. The rate of damage and weight loss of the material depends on how the pest attacks or destroys it. If pest attacks
continue, it will cause a decrease in quality and contamination which will result in food unfit for consumption (Rizal, 2019).

The primary pest that damages rice is the beetle *Sitophilus oryzae* L. Rice beetle is a type of warehouse insect pest that attacks rice, thereby reducing its quality in rice storage areas. *S. oryzae* causes rice grains to become hollow and easily crumble like flour (Rizal, 2019). The relationship between *S. oryzae* population density and population growth, the percentage of weight loss, the percentage of hollow rice, the percentage of rice powder, and changes in water content increase linearly (Hendrival & Melinda, 2017). In addition, it causes rice to be unpleasant and smells bad, thereby reducing the quality of rice and causing economic losses (Booroto et al., 2017).

Botanical pesticides are pesticides whose raw materials come from plants and contain active ingredients that can control insect pests (Isnaini et al., 2015). Plants contain chemical compounds in the form of secondary metabolites which play a role in the process of interaction and competition. The products of these secondary metabolites are used as active ingredients in vegetable pesticides. Some of the secondary metabolites in plants are volatile compounds, such as essential oils, citral, geraniol, tannins, piperine, acetogenins, azadirachtin, saponins, asaron, akoragermakron, akolamonin, isoakolamin, calameon, kalamediol, alfamirin, and several groups of acids, such as cyanide acid, oleanolic acid, and galoyonic acid (Saenong, 2016).

Essential oils are vegetable oils that have several characteristics, namely they are easy to evaporate, have the same aromatic smell as the plant they come from, and have a bitter taste. Essential oil is also called flying oil. Essential oils are obtained from several parts of plants, such as leaves, fruit, seeds, flowers, roots, rhizomes, and bark (Andila et al., 2020).

Nutmeg contains 16-17% essential oil, while nutmeg mace has an essential oil content of 4-15%. The taste characteristics of nutmeg are fresh, bitter, warm, spicy, sweet, and have a sharp aroma. The specific components of nutmeg are cineole and *α*-terpinyl acetate (Anto, 2020).

Clove plant is a plant that has the potential to produce essential oils. The parts of the clove plant that can produce essential oil are the flowers, stalks and leaves. In the flower section, the essential oil content is 21.3% with a eugenol content of 78-95%. In the stalk, the essential oil content is 6% with a eugenol content of 89-95%, while the leaves contain an essential oil of 2-3% with a eugenol content of 80-85%. Eugenol is the main ingredient of clove oil which is useful in giving aroma to perfumes, food and drinks (Hadi, 2012).

Therefore, the aim of this study was to determine the inhibition of the development of the F1 generation of the rice beetle (*Sitophilus oryzae* L.) by the essential oils of nutmeg (*Myristica fragrans* Houtt.) and clove flower (*Syzygium aromaticum* L.), and to determine the mortality of the rice beetle. (*Sitophilus oryzae* L.) on essential oils of nutmeg (*Myristica fragrans* Houtt.) and clove flowers (*Syzygium aromaticum* L.).
METHODS

This research was carried out from January 2022 to May 2022 at the Animal Ecology and Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda. The research was conducted using two methods. Activity method of inhibiting F1 generation of rice beetles and contact method with filter paper media.

Research Stages
1. Rearing Rice Beetle (S. oryzae)
   According to Lestari (2020), rearing insects begins with 2000 grams of rice being put into three large 5L volume jars which will be used as media and feed for the mass propagation of rice beetles. This propagation is done with a minimum of 50 male imago and 50 female imago placed in each jar. Then, the jar is covered with a chiffon cloth. Imago is obtained from purchasing rice that has rice beetles. Differences between male and female imagos were seen using a magnifying glass (lup) based on the size of the imago's body. The male imago has a shorter and slender rostrum size and its abdomen when viewed from the lateral and posterior has a curved and tapered shape. The female imago has a longer and bigger rostrum size, and its abdomen when viewed from the lateral and posterior has a straight and enlarged shape.

2. Essential Oil
   Essential Oils of Nutmeg (Myristica fragrans Houtt.) and Clove Flowers (Syzygium aromaticum L.) are obtained from the production of Serambi Botany.

3. Rice Beetle F1 Generation Inhibition Activity
   First of all, tools and materials are prepared. Then, 50 grams of rice is put into a plastic cup. After that, 1 mL of each essential oil dose was taken and dripped into the rice in a plastic cup. Then, flatten and dry the feed for 5 minutes. After that, 10 Sitophilus oryzae L. imago aged 5-10 days were infested into the plastic cup. Furthermore, the plastic cup is covered with a plastic cup cover, the middle of which has been given a chiffon cloth. On the 4th day, all the insects in the plastic cup were transferred to another container. Then, in plastic cups it was observed for 35 days to 50 days until there was an F1 generation with 3 repetitions (Ainun, 2019).

   Observation of inhibitors of the development of the F1 generation of Sitophilus oryzae L. using the formula below:

   \[
   \text{Percentage} = \frac{(\text{NC} - \text{NT})}{\text{NC}} \times 100\%
   \]

   Annotation:
   \(\text{NC} = \) The number of imago contained in the control
   \(\text{NT} = \) The number of imago contained in the treatment

4. Contact method with Filter Paper media
   One (1) mL of each essential oil dose for each treatment was taken using a pipette. Then, the essential oil was dripped evenly on filter paper which has a diameter of 9 cm in a petri dish. Then, the filter paper was dried for 5 minutes. After that, a pinch of rice was added and 10 Sitophilus oryzae L. imago were infested in a petri dish. Next, the petri dish was closed. After that, it was carried out
for 24 hours, 48 hours and 72 hours with 3 repetitions (Ainun, 2019).

Insect mortality

\[ P_0 = \frac{r}{n} \]

Annotation:

\( P_0 \) = Insect mortality (%)

\( r \) = Number of insects die

\( m \) = Number of insects observed

Data Analysis

The data analysis technique used in this study is SPSS. If the data is homogeneous, then the One Way Analysis of Variance (ANOVA) test is performed. Then, if the results found are significantly different with a confidence interval \( P < 0.05 \), then proceed with the Tukey test. If the data is not homogeneous and not normal, then the data analysis uses non-parametric, namely Kruskal Wallis, whereas if the results are significantly different, then it is continued with the Mann-Whitney test (Endra, 2017)

RESULTS AND DISCUSSION

Inhibition of Rice Beetle F1 Generation (Sitophilus oryzae)

The results of observing the F1 generation inhibition method of the rice weevil (Sitophilus oryzae) carried out for 50 days on nutmeg (M. fragrans) (MAP) and clove flower (S. aromaticum) (MAC) essential oils are in table 1 and table 2.

Table 1. Percentage of inhibition of F1 generation of rice beetles using clove flower essential oil (S. aromaticum) after 50 days of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inhibition of Rice Beetle F1 Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td>Control</td>
<td>0(^a)</td>
</tr>
<tr>
<td>MAC 2.5%</td>
<td>43.00 ± 24.98(^b)</td>
</tr>
<tr>
<td>MAC 5%</td>
<td>65.00 ± 17.32(^b)</td>
</tr>
<tr>
<td>MAC 10%</td>
<td>69.67 ± 26.50(^b)</td>
</tr>
<tr>
<td>MAC 20%</td>
<td>90.33 ± 13.42(^b)</td>
</tr>
</tbody>
</table>

Annotation : Numbers followed by the same letter in the same column are not significantly different, while letters that are not the same are significantly different on the Tukey test at a significance level of 5% (\( P<0.05 \))

The results of observations (Table 1 and Table 2) showed that the inhibition of F1 generation in all treatments was significantly different from the water control and water + Tween-80 controls. Inhibition of the F1 generation of rice beetles in all treatments showed a value of more than 50%. Nutmeg oil and clove oil can be used to inhibit the development of F1 rice beetles. However, the highest effectiveness in inhibiting the development of the rice beetle F1 generation was nutmeg oil at concentrations of 5%, 10%, and 20%. This is presumably because there is inhibition in female insects when laying their eggs, so that egg laying is
inhibited. This statement is in accordance with Mulyani et al. (2021) which states that, there are five ways insecticides work, namely affecting the nervous system, inhibiting energy production, affecting the endocrine system, inhibiting cuticle production and inhibiting water balance. Insecticides can enter the insect's body through the cuticle, digestive organs, and respiratory holes. Hasyim (2014) states, the effect of vegetable insecticides is inhibition of laying by insect pests as a result of disruption of female insect receptors which is a combination of responses to visual, mechanical, and olfactory and gustatory stimuli to lay eggs.

Table 2. Percentage of inhibition of F1 generation in rice beetles using nutmeg (M. fragrans) essential oil after 50 days of treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water</th>
<th>Water+Tween-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0a</td>
<td>0a</td>
</tr>
<tr>
<td>MAP 2,5%</td>
<td>75,00 ± 8.71b</td>
<td>66,00 ± 2.64b</td>
</tr>
<tr>
<td>MAP 5%</td>
<td>100 ± 0.00c</td>
<td>100 ± 0.00c</td>
</tr>
<tr>
<td>MAP 10%</td>
<td>100 ± 0.00c</td>
<td>100 ± 0.00c</td>
</tr>
<tr>
<td>MAP 20%</td>
<td>100 ± 0.00c</td>
<td>100 ± 0.00c</td>
</tr>
</tbody>
</table>

Annotation: Numbers followed by the same letter in the same column were not significantly different, while letters that were not the same indicated a significant difference on the Tukey test at a significance level of 5% (P<0.05).

The life cycle of the rice beetle consists of four stages, namely egg, larva, pupa and adult. Giving nutmeg oil and clove oil to rice is thought to inhibit one of the four stages in the life cycle of the rice beetle, so it does not grow and develop. This is in accordance with Eka et al. (2018) which stated that the saponins found in cloves and nutmeg are able to inhibit the growth of insects by interfering with the molting stage of the larvae. In addition, saponins cause changes in membrane permeability. According to Nuraeni & Darwiati (2021) saponins are the cause of decreased work of digestive enzymes and food absorption due to interactions between saponins and mucous cell membranes, this interaction causes loss of enzyme binding activity in membranes to changes in cell membrane permeability.

Disruption of larval growth to failure of metamorphosis of rice beetles is thought to be the cause of the little to no imago formed in the treatment with nutmeg and clove oil. This statement is in accordance with Maheswari et al. (2018) one of the causes of the disruption of the growth of larvae from insects is alkaloids. Alkaloids are capable of acting as stomach poisons, so if they enter the body of the larvae they will disrupt the digestion of the larvae of the rice beetle causing death. According to
Nuraeni & Darwiati (2021) alkaloids are able to inhibit the growth of insects because of the nature of the alkaloids in the form of salts, resulting in degradation of cell membranes and inhibiting the action of the acetyl cholinesterase enzyme. Inhibition of the action of these enzymes causes disruption of the workings of the larval nervous system.

Figure 1. Graph of mean comparison between treatments and controls on the inhibition of the rice beetle F1 generation.

Based on the results of the graph (Figure 1) it is generally shown that clove oil and nutmeg have an effect on inhibiting the development and growth of the F1 generation of rice beetles. This is presumably due to the insecticidal properties of nutmeg oil and clove oil against rice beetles. This statement is in accordance with Mulyani (2021). Essential oils have broad activity in preventing and killing insects, but are relatively non-toxic to the environment. Some of the activities of essential oils are as an antifeedant, preventing egg laying, and growth regulators. The comparison value with the water control is higher than the water+tween-80 control. However, relatively constant 100% inhibition on 5%, 10%, and 20% nutmeg oil. This is presumably because water control has no effect on inhibiting the growth and development of rice weevils, while water+tween-80 is an emulsifier which has an effect on rice water content. So, in comparison with water control there is a difference. This statement is in accordance with Wikantyasning & Indianie (2021), Tween-80 is a water-soluble emulsifying agent. Tween-80 is hydrophilic which will bind to the water phase, so that more water molecules will be attracted.

Based on table 3, the average number of rice beetles that lived after being given the control and essential oil treatments with three repetitions obtained the results with the highest average in the water control, then
followed by the water control + tween-80, and the treatment. This is presumably because the water control did not affect the inhibition of the F1 generation of rice beetles, while the treatment had an effect along with the concentration given to the rice. This statement is in accordance with Maheswari et al. (2018) giving inactive treatments at low concentrations can be caused by the inactivity of chemical compounds found in nutmeg and clove oils. The higher the concentration used the more chemical compounds will be. Thus, the higher the toxicity.

Table 3. The average number of rice beetles that lived after being treated with nutmeg and clove essential oils

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Number of Lives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Control</td>
<td>93.67</td>
</tr>
<tr>
<td>Water Control + Tween-80</td>
<td>71.00</td>
</tr>
<tr>
<td>MAC 2.5%</td>
<td>54.67</td>
</tr>
<tr>
<td>MAC 5%</td>
<td>36.00</td>
</tr>
<tr>
<td>MAC 10%</td>
<td>33.00</td>
</tr>
<tr>
<td>MAC 20%</td>
<td>11.33</td>
</tr>
<tr>
<td>MAP 2.5%</td>
<td>24.67</td>
</tr>
<tr>
<td>MAP 5%</td>
<td>0.00</td>
</tr>
<tr>
<td>MAP 10%</td>
<td>0.00</td>
</tr>
<tr>
<td>MAP 20%</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Rice Beetle Paper Filter Method (Sitophilus oryzae)

The results of observations of the rice beetle filter paper method carried out for 24 hours, 48 hours, and 72 hours for the essential oils of nutmeg (M. fragrans) and clove flowers (S. aromaticum) are shown in table 4 and table 5 as follows.

Table 4. Percentage of deaths using filter paper method of rice beetle (Sitophilus oryzae) using clove flower essential oil (S. aromaticum)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
</tr>
<tr>
<td>Water + Tween-80</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
</tr>
<tr>
<td>MAC 2.5%</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
</tr>
<tr>
<td>MAC 5%</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
<td>$0 \pm 0.00^a$</td>
</tr>
<tr>
<td>MAC 10%</td>
<td>$3.33 \pm 5.77^a$</td>
<td>$13.33 \pm 5.77^b$</td>
<td>$20.00 \pm 10.00^b$</td>
</tr>
<tr>
<td>MAC 20%</td>
<td>$63.33 \pm 35.11^b$</td>
<td>$93.33 \pm 11.54^c$</td>
<td>$96.67 \pm 5.77^b$</td>
</tr>
</tbody>
</table>

Annotation: Numbers followed by the same letter in the same column were not significantly different, while letters that were not the same indicated a significant difference in the Mann-Whitney test at a significance level of 5% (P<0.05).

Observations (Table 4 and Table 5) show that the mortality rate at 24 hours can kill rice beetles by up to 50%, this is presumably because
nuttmeg and clove oil reduce the resistance of rice beetles because they contain various compounds that are toxic to them. Pamungkas et al. (2017) which stated that cloves contain several chemical substances, one of which is flavonoids. The content of flavonoids in cloves will affect the nervous system in insects, causing death. According to Astuthi et al. (2017) nutmeg essential oil has antifeedant properties which prevent insects from consuming the plants sprayed by the essential oil.

Table 5. Percentage of deaths using filter paper method of rice beetle (Sitophilus oryzae) using essential oil of nutmeg (M. fragrans).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24 Hours</th>
<th>48 Hours</th>
<th>72 Jam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0 ± 0.00a</td>
<td>0 ± 0.00a</td>
<td>0 ± 0.00a</td>
</tr>
<tr>
<td>Water + Tween-80</td>
<td>0 ± 0.00a</td>
<td>0 ± 0.00a</td>
<td>0 ± 0.00a</td>
</tr>
<tr>
<td>MAP 2.5%</td>
<td>56.67 ± 5.77b</td>
<td>96.67 ± 5.77b</td>
<td>100 ± 0.00b</td>
</tr>
<tr>
<td>MAP 5%</td>
<td>96.67 ± 5.77c</td>
<td>100 ± 0.00b</td>
<td>100 ± 0.00b</td>
</tr>
<tr>
<td>MAP 10%</td>
<td>100 ± 0.00c</td>
<td>100 ± 0.00b</td>
<td>100 ± 0.00b</td>
</tr>
<tr>
<td>MAP 20%</td>
<td>100 ± 0.00c</td>
<td>100 ± 0.00b</td>
<td>100 ± 0.00b</td>
</tr>
</tbody>
</table>

Annotation : Numbers followed by the same letter in the same column were not significantly different, while letters that were not the same indicated a significant difference in the Mann-Whitney test at a significance level of 5% (P<0.05).

In observation after 48 hours, nutmeg oil was able to kill rice beetles starting at a concentration of 2.5% - 20%, while clove oil was able to kill from a concentration of 10% -20%. The results of observations for 72 hours showed that nutmeg oil was able to kill rice beetles up to 100% at all concentrations, while cloves were able to kill up to 90% in 72 hours at 20% concentration. The higher the dose of essential oil used, the higher the percentage of deaths from rice beetles. This is presumably due to the presence of saponins contained in nutmeg and clove oil. This statement is in accordance with Ariwidiani et al. (2021) saponins, which are chemical constituents of nutmeg and clove oil, cause a decrease in the surface tension of the mucous membranes of the digestive tract, so that the tracheal walls become corrosive. In addition, saponins can damage cell membranes and even disrupt metabolism, causing death through the respiratory organs.

The results of treatment with three observation times, nutmeg oil has a higher effectiveness than clove oil. Seen in the high percentage of death at all concentrations of nutmeg oil. It is suspected that there is a myristin content in nutmeg which is toxic to insects. This statement is in accordance with Rahman et al. (2015) myristin can be a cause of neurotoxicity. Neurotoxicity is caused by exposure to chemicals and causes other nerve damage.

Another chemical substance contained in nutmeg oil which is thought to be the cause of the high percentage of deaths in the administration of nutmeg oil to rice
weevils is the terpenoid compound in nutmeg oil. This statement is in accordance with Purwatiningsih et al. (2019), terpenoids have a distinctive odor, so insects don't like them. In addition, terpenoids affect nerve function and reduce muscle work, leading to death in insects.

The effect of nutmeg oil on rice beetles was seen at a concentration of 2.5% for the first 24 hours with an effectiveness above 50%. Effectiveness increases with increasing concentration of nutmeg oil. At 48 hours, the average mortality was above 90% with a concentration of 2.5% nutmeg oil, up to 72 hours of observation, constant mortality for all treatments with nutmeg oil had 100% effectiveness. This statement is in accordance with Ainun (2019) that the increase in the concentration of essential oils is directly proportional to the high poison given to insects, so the killing power of essential oils is higher.

![Figure 2. Graph of the average comparison of each treatment for 24 hours, 48 hours and 72 hours.](image)

Based on the graph (Figure 2) the effect of clove oil on rice beetles was only seen at a concentration of 10%. This is presumably because the eugenol content in clove oil can affect its concentration in rice beetles. The statement complies with Baker & Grant. (2018), eugenol has contact activity by disrupting cell membranes. The reaction is in line with the dose given. If the dose is low, eugenol acts as an attractant. In addition, this statement is in accordance with Chinthia (2015), eugenol can affect the nervous system, so it can cause death in insects.

The death of rice beetles by clove oil treatment can be caused by disruption of the feeding mechanism, so that the digestive process of rice beetles is disrupted. This statement is in accordance with Eka & Triastinurmatingsih (2018) which states that cloves contain tannins.
which have a bitter taste, resulting in inhibition in the digestive process. Tannins will bind to proteins in the natural digestive system needed by insects in their growth.

CONCLUSION AND RECOMMENDATION

From the research that has been done, it can be concluded that nutmeg oil (*Myristica fragrans* Houtt.) and clove oil (*Syzygium aromaticum* L.) can inhibit the F1 generation of rice beetles starting from a concentration of 2.5% to a concentration of 20% with % inhibition of 43% to 100%. Nutmeg Oil is more effective because it can inhibit up to 100%.

Nutmeg oil (*Myristica fragrans* Houtt.) within 24 hours can kill rice beetles with a concentration of 20% with a mortality of up to 100%. whereas clove oil was only able to kill rice weevils in the same time span and concentration with a mortality of 63%. Until now there has been no research report on the effect of essential oils used on the quality of treated rice.

The suggestion for further research is to obtain more complete data/references, it is advisable to test various other essential oils. Once complete, research on the effect of essential oils on the quality of treated rice can be carried out.

REFERENCES


The Potensial of Nutmeg Oil (Myristica fragrans Houtt.) and Clove Flower (Syzygium aromaticum L.) as Insecticide on Mortality and Development Rice Beetle (Sitophilus oryzae L.) (Linnaeus, 1763)


