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Effectiveness of Legundi Leaf Powder Seed Coating (*Vitex trifolia* L.) on Rice Seeds Against Rice Weevil (*Sitophilus oryzae* L.) Mortality

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the main food commodities in Indonesia. However, rice production has declined in recent years. One factor contributing to this decline is seed deterioration. During storage, rice seeds are commonly infested by rice weevil (*Sitophilus oryzae* L.). One solution to this problem is the application of seed coating technology using botanical insecticides. This study aimed to develop seed coating technology using legundi leaf powder and evaluate its effect on *S. oryzae* mortality. The study used a completely randomized design consisting of five legundi leaf powder dose treatments (D) and one control, namely D0: control, D1: 5 g/50 g seeds, D2: 7.5 g/50 g seeds, D3: 10 g/50 g seeds, D4: 12.5 g/50 g seeds, and D5: 15 g/50 g seeds. The observed variables were *S. oryzae* mortality, seed germination, and maximum seed growth potential. The results showed that treatment D5 produced the highest *S. oryzae* mortality (80%), which was significantly different from treatments D0, D1, D2, and D3, but not significantly different from treatment D4 (50%). The application of different doses of legundi leaf powder had no significant effect (ns) on seed germination and maximum growth potential.

Keywords: *seed coating, V. trifolia leaf powder, rice seeds, mortality, S. oryzae*

INTRODUCTION

Rice (*Oryza sativa* L.) is a strategic commodity in the national food system because it serves as the main source of carbohydrates for most of the Indonesian population. High rice consumption has not been fully balanced by rice production, which has tended to decline. Based on data from Statistics Indonesia (2025, data for 2002-2024), rice production reached 54,748,977.00 tons in 2022, 53,980,993.19 tons in 2023, and 53,142,726.65 tons in 2024 (final figure). This decline in rice production has been influenced by significant climate change, particularly prolonged drought, which has resulted in planting failure and crop failure (Badan Pusat Statistik Kabupaten Pati, 2023). In addition to field-related factors, declining rice production can also be traced to the storage stage, particularly due to storage pest infestation, which reduces the quantity and quality of grain. During storage (postharvest), seed deterioration occurs rapidly; consequently, the availability of high-quality seed decreases and affects production output (Nazilah & Suharjono, 2021).

During storage, rice seeds are commonly attacked by storage pests, particularly rice weevil (*Sitophilus oryzae* L.), after being stored for one month or longer, thereby reducing rice seed quality. Rice weevil (*S. oryzae*) causes damage characterized by holes in rice seeds and seeds turning into powder (Mastuti *et al.*, 2020). Seeds have a seed coat that functions to protect the embryo or internal seed tissues, regulate the rate of water uptake, regulate the entry of oxygen, carbon dioxide, and other gases, control germination, and induce dormancy (SITH ITB, 2016). Rice weevil feeds on and lays eggs in rice seeds by creating small holes with its snout to a depth of approximately 1 mm. In addition, the larvae form cocoons or protective chambers for the pupae by excreting fluid onto the endosperm walls, making the walls smooth and creating a strong texture (Talib, 2018). During the adult phase, rice weevils still inside the seed emerge by making relatively large holes with their snouts. Damage to the seed structure directly affects the germination process. Farmers are disadvantaged by this condition because of the high potential for reduced seed viability and production yield. A solution that can be applied to maintain rice seed quality from rice weevil (*S. oryzae*) attack during storage is seed coating technology using botanical insecticides derived from specific plant parts.

Seed coating is performed by covering the entire seed surface with a coating agent

that adheres perfectly to the seed surface without altering seed shape (Yuli, 2017). Seed coating is considered effective because this seed performance enhancement technique not only improves seed appearance but can also increase seed storability and reduce the risk of pest and disease infection from the surrounding environment (Natasi, 2021). One material used in seed coating technology is polymer. Commercial polymers used as seed coatings are usually combined with active ingredients such as synthetic pesticides to prevent seed damage caused by pathogens or pests. However, long-term use of synthetic pesticides can cause environmental pollution. A more environmentally safe active ingredient alternative for seed coating is the use of botanical pesticides.

The use of botanical insecticides has several advantages, including rapid degradation of active compounds in the ecosystem, greater specificity compared with synthetic insecticides, and suppression of pest resistance to insecticides. One plant species with potential as a botanical insecticide for seed coating is legundi.

Legundi (*Vitex trifolia* L.) has a distinctive spice-like aroma. The leaves are the plant part most commonly used as a botanical insecticide. Several active compounds are contained in legundi leaves, including flavonoids, alkaloids, polyphenols, saponins, and essential oils (Khairul and Vemithasa, 2018). A study by Jayuska *et al.* (2022) showed that legundi leaves (*V. trifolia*), through their essential oil content, were effective as a repellent against rice weevil (*S. oryzae*). In addition, Khairul and Vemithasa (2018) reported that ethyl acetate extract of *V. trifolia* leaves showed a strong effect on *C. pavonana* larvae; the best concentration (0.50%) of legundi leaf ethyl acetate extract caused larval mortality of 85.33%.

Based on previous studies, legundi leaves have been widely tested as botanical insecticides for pest control. However, few studies have evaluated the effectiveness of legundi leaves when applied in seed coating technology. In this study, legundi leaf powder was used as the seed coating material because powder or flour forms have been proven to be more capable of controlling storage pests, efficient in terms of time and labor, and more economical than other control methods. The dry powder form also minimizes the possibility of microorganism attack, such as fungi and bacteria (Makuku *et al.*, 2022).

Based on this background, the objective of this study was to develop seed coating technology using legundi leaves on rice seeds and to evaluate its effect on the mortality rate of *S. oryzae* and seed viability. This study is expected to contribute to the development of environmentally friendly technology for postharvest pest control while remaining safe for seed germination.

MATERIALS AND METHODS

Time and Place of Research

This study was a semi-field experimental study conducted from March to July 2025. The research was carried out at the Agricultural Biotechnology Laboratory, Udayana University. Young leaf samples of legundi plants were collected from Sambangan Village, Sukasada District, Buleleng Regency. Adult rice weevils and certified BP-class Inpari 32 HDB rice seeds were obtained from a rice seed grower in Subak Delod Sema, Sading Village, Mengwi District, Badung Regency.

Tools and Materials

The tools used in this study included a blender, 200-mesh sieve, jars, forceps, analytical balance, gauze cloth, rubber bands, label paper, zip-lock plastic bags, thinwall containers, tissue paper, sprayer, stationery, and camera. The materials used in this study were legundi leaves (coating material), distilled water (solvent), Inpari 32 HDB rice seeds with a moisture content of 11.8%, adult rice weevils (males and females) from contaminated rice seeds, 1.25 g xanthan gum (adhesive) for 50 g seeds, and 0.625 g pectin (adhesive) for 50 g seeds.

Treatments and Experimental Design

This study used a completely randomized design (CRD) consisting of five dose treatments of legundi leaf powder (D1: 5 g/50 g seeds, D2: 7.5 g/50 g seeds, D3: 10 g/50 g seeds, D4: 12.5 g/50 g seeds, and D5: 15 g/50 g seeds) and one untreated treatment (D0: control). Each treatment was repeated four times, resulting in 24 experimental units with 200 g of seeds per replicate. The observed variables were *S. oryzae* mortality, seed germination, and maximum seed growth potential.

Sample Collection

At the initial stage of the study, fresh legundi leaves were collected from Sambangan Village, Buleleng. The collected leaf samples were then brought to the laboratory and cleaned under running water. The leaves were subsequently air-dried in an open container for approximately seven days.

Preparation of Legundi Leaf Powder

Dried legundi leaf samples were blended to obtain fine powder. The legundi leaf powder was then sieved using a mesh sieve to obtain a fine powder. The fine legundi leaf powder was subsequently stored in airtight jars at room temperature.

Preparation of Test Insects

Adult rice weevils were collected from stored infested rice seeds. Subsequently, 20 pairs of male and female adults were transferred into a rearing container, namely a plastic jar filled with 500 g of rice seeds as feed, and the container was covered with gauze cloth. The rice weevils were reared for approximately 40 days to produce young and uniform F1 rice weevils. Rearing was conducted in a room not exposed to light at a temperature of approximately 29°C. Young (virgin) *S. oryzae* were characterized by a brown to slightly reddish color.

Preparation of Seed Coating on Rice Seeds

A total of 1.25 g xanthan gum and 0.625 g pectin were placed in one container and dissolved in 25 mL of water or distilled water. The mixture was stirred until homogeneous and thickened. Then, 50 g of rice seeds were added to the mixture until all seed surfaces were coated. The coated seeds were then further coated with legundi leaf powder according to the treatment doses. The rice seeds coated with legundi leaf powder according to the treatment doses were air-dried for +/- 24 hours, stored in zip-lock plastic bags, and labeled according to each treatment.

Pest Starvation

Adult rice weevils that had been previously reared were separated from the rearing jars. Then, five *S. oryzae* individuals were placed into each test jar, resulting in a total of 120 pests used in the assay. Before being infested with treated seeds, the pests were first starved or not given feed for approximately five hours in the test jars to increase feeding activity during infestation.

***S. oryzae* Infestation**

After five hours of starvation, the next step was pest infestation in the test jars. *S. oryzae* infestation was initiated by first removing the pests that had previously been starved in the test jars. Each test jar was then filled with 50 g of seeds according to the treatment or control. Five *S. oryzae* individuals were then introduced into each test jar containing rice seeds. Each test jar was then covered with gauze cloth. The test jars were placed in a room at approximately 29°C and with limited light exposure.

Observation of *S. oryzae* Mortality

S. oryzae mortality was observed at 12, 24, 36, 48, 60, and 72 hours after infestation. In each treatment jar, the number of dead *S. oryzae* adults was observed and counted. *S. oryzae* mortality was calculated using the following formula (Junaedi, Yunus, and Edy, 2024):

$$P = \frac{a}{b} \times 100\% \qquad (1)$$

Note:
P = Mortality percentage of *S. oryzae*
a = Number of dead *S. oryzae*
b = Number of tested *S. oryzae*

Seed Germination Test

The seed germination test used 100 rice seeds from each treatment and control. The method applied in this study was the top-of-paper test using tissue paper as the substrate. Three sheets of paper were placed in a thinwall container and then moistened using a sprayer filled with distilled water. Next, 100 rice seeds were arranged regularly and evenly spaced on the paper in a horizontal position, and the container was closed to maintain humidity.

Seed germination was calculated using the following formula (Wijana, 2024):

$$\text{DB (\%)} = \frac{\text{Normal seedlings count 1} + \text{count 2}}{\text{Seeds sown}} \times 100\% \qquad (2)$$

Note:
DB = Seed Germination
Counts 1 and 2 differ depending on seed type. For rice seeds, the first count was conducted at five days after sowing and the second count at 14 days after sowing.

Observation of Maximum Seed Growth Potential

Maximum growth potential is the number of seeds that remain alive or show signs of life, including seeds that grow normally and abnormally, and is expressed as a percentage (%) (Jahrotul, 2023). Maximum seed growth potential was obtained using the following formula (Wijana, 2024):

$$\text{PTM } (\%) \frac{(\text{normal} + \text{abnormal seedlings})}{\text{seeds sown}} \times 100\% \qquad (3)$$

Note:
PTM = Maximum Growth Potential

Data Analysis

The data obtained from observations were analyzed using analysis of variance (ANOVA). When a significant effect was detected, further testing was conducted using Duncan's Multiple Range Test (DMRT) at the 5% level.

RESULTS AND DISCUSSION

S. oryzae Mortality

S. oryzae mortality was observed for three days at 12-hour intervals by recording the number of pests that died in each treatment. The indicator used to confirm that rice weevils had died was the absence of movement in insect body parts such as the antennae, legs, wings, and body. Another indicator was body color; insects that died after treatment had a reddish-brown color, whereas rice weevils in the control were reddish black.

Figure 1 shows that the highest mortality percentage occurred in D5, reaching 80% at the final observation or 72 hours after infestation, followed by D4 with a mortality percentage of 50%. The results of Duncan's Multiple Range Test (DMRT) at the 5% level for rice weevil mortality after 72 hours of treatment are presented below.

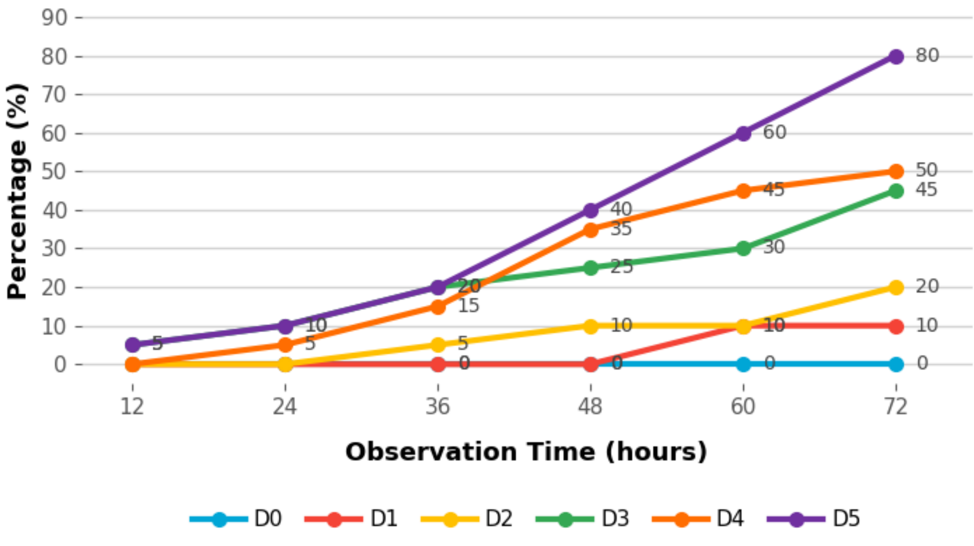

Figure 1. Mortality percentage of rice weevil pests (*S. oryzae*)

Table 1. Mortality level of S. oryzae

Dose of legundi leaf powder	Mortality (%)
Control (D0)	0 c
5 g (D1)	10 c
7.5 g (D2)	20 bc
10 g (D3)	45 b
12.5 g (D4)	50 ab
15 g (D5)	80 a

Note: Values followed by the same letter in the same column indicate no significant difference according to Duncan's Multiple Range Test (DMRT) at the 5% level.

The test results in Table 1 demonstrate that treatment D5 produced the highest *S. oryzae* mortality of 80%, which was significantly different from treatments D0, D1, D2, and D3, but not significantly different from treatment D4, which reached 50%. Thus, the toxic exposure received by the insect pests was relatively high because the insecticide dose used was also higher than in the other treatments. Although statistically not significantly different from D4, treatment D5 was more effective in practical terms because the difference in mortality rate was relatively large, namely 30%, and the rice weevil mortality percentage exceeded 50%. According to Isnaini *et al.* (2015), a botanical insecticide can be considered effective when rice weevil mortality is >50%. Treatments D1, D2, and D3 were considered ineffective because *S. oryzae* mortality did not reach 50%.

Legundi leaves have been proven to contain various secondary metabolite compounds that can function in defense against pests such as *S. oryzae*. The compounds in legundi leaves include phenolic compounds such as tannins, which are toxic because they cause digestive disorders in pests and inhibit pest feeding activity (antifeedant) (Lina, 2016). Similarly, alkaloid and flavonoid compounds enter the bodies of test pests through consumed food or act as stomach poisons, thereby disrupting the digestive system of the pests and causing pest death (Yogantara *et al.*, 2017). Legundi leaves also contain essential oils belonging to the terpenoid compound group, which produce the characteristic aroma of the leaves and cause the test pests to fail to recognize their food and become reluctant to feed (antifeedant effect) (Lina, 2016). According to Jayuska *et al.* (2022), five main essential oil compounds are contained in legundi leaves, namely beta-pinene, trans-caryophyllene, cyclohexanol, beta-ocimene, and eucalyptol. The active compounds in legundi leaves trigger responses in insect sensory organs, resulting in reduced or halted feeding activity (Khairul & Vemithasa, 2018).

However, the antifeedant effect did not immediately stop the feeding activity of the test pests in this study. This was due to the starvation process applied to the pests before they were infested into the test jars. The starvation process caused the pests to continue feeding on seeds with coating treatments; therefore, pest mortality occurred in jars containing coated seeds. In contrast, no pest mortality was observed in the control jars (D0) until the end of the observation period (0%). Higher doses strengthened the effects of various active compounds in legundi leaf powder; therefore, D5 and D4 showed higher pest mortality.

Seed Germination

The seed germination test was conducted by testing a number of seeds and calculating the percentage of total seeds that successfully grew or germinated properly within the predetermined period (Jahrotul, 2023). The analysis of variance showed that several doses of legundi leaf powder as a seed coating material for rice seeds had no significant effect (ns) on seed germination. Based on the germination test, both control seeds and treated seeds (D1, D2, D3, D4, and D5) showed high germination. The germination standard categorized as high for most seed types is >=80% (Nazilah & Suharjono, 2021).

The highest germination was found in seeds treated with seed coating using 12.5 g of legundi leaf powder (D4), namely 94%, followed by D5, D3, D2, D1, and D0 (Table 2). Based on the germination standard, all treatments still had high germination. Differences in germination among treatments may be caused by various factors, such as external microorganisms, temperature, humidity, and seed condition.

Seed coating with legundi leaf powder is presumed not to directly function as a substance that stimulates the germination process. However, the results showed that seed coating with legundi leaf powder could maintain seed viability (Figure 2). Seed coating treatments even slightly increased seed germination, as seen from the comparison with D0 germination (92%), whereas D3 and D5 reached 93% and D4 reached 94%. However, this increase was not statistically significant. This condition may occur because the polymer layer and legundi leaf powder covering the seeds protect the seeds from pests and pathogens in the surrounding environment. Xanthan gum is a polymer that forms a rigid layer, and its combination with pectin increases seed permeability, so that the seeds are protected by a strong layer while water imbibition into the seeds can still occur (Hazirah *et al.*, 2016). Therefore, seed coating is effective in providing protection from pests and remains safe for seed viability.

Maximum Seed Growth Potential

Maximum seed growth potential is also a parameter of seed viability. The difference from germination is that the number of living seeds counted includes not only normally germinated seeds but also abnormal seedlings. Maximum seed growth potential was measured at the end of the observation or at the second count, namely 14 days after sowing.

Based on the analysis of variance, several doses of legundi leaf powder as a seed coating material for rice seeds had no significant effect (ns) on maximum seed growth potential. The test results showed that the highest maximum growth potential (%) was found in treatment D3, at 95%, followed by D0 (94%), D4 (94%), D5 (93%), D2 (93%), and D1 (93%) (Table 3). The maximum growth potential of each treatment was classified as high; therefore, legundi leaf powder seed coating was proven to maintain seed viability.

It can be seen that botanical insecticide treatment as seed coating does not always increase growth potential but tends to maintain seed quality within the same range as the control. Maximum growth potential values above 80% indicate that the seeds are in good condition and suitable for planting, even though no significant increase occurred as a result of the treatment.

Table 2. Seed Germination Percentage

Treatment	Seed Germination (%)
Control (D0)	92 a
5 g (D1)	92 a
7.5 g (D2)	92 a
10 g (D3)	93 a
12.5 g (D4)	94 a
15 g (D5)	93 a

Table 3. Percentage of Maximum Seed Growth Potential

Treatment	Maximum Growth Potential (%)
Control (D0)	94 a
5 g (D1)	93 a
7.5 g (D2)	93 a
10 g (D3)	95 a
12.5 g (D4)	94 a
15 g (D5)	93 a

Figure 2. Viability of rice seeds with coating and without coating

CONCLUSION

The most effective dose of legundi leaf powder as a seed coating material on rice seeds against *S. oryzae* mortality was 15 g/50 g rice seeds, with pest mortality reaching 80%, and this was not significantly different from the dose of 12.5 g/50 g seeds. The results of the germination test and maximum seed growth potential test demonstrated that the viability of treated and control seeds was high, namely >=80%. Further studies can be conducted to evaluate the effect of seed storage duration on the quality of seeds treated with this seed coating.

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