



## Efficacy of *Vitex trifolia* Leaf Powder as a Sustainable Botanical Insecticide Against the Rice Weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae)

Muhammad Sayuthi<sup>\*</sup>, Susanna, Farhas Alfarisy

Faculty of Agriculture, Department of Plant Protection, Syiah Kuala University, Banda Aceh 23111, Indonesia

Corresponding Author Email: [say\\_m2001@usk.ac.id](mailto:say_m2001@usk.ac.id)

Copyright: ©2026 The authors. This article is published by IETA and is licensed under the CC BY 4.0 license (<http://creativecommons.org/licenses/by/4.0/>).

<https://doi.org/10.18280/ij dne.210117>

### ABSTRACT

**Received:** 12 November 2025

**Revised:** 17 January 2026

**Accepted:** 26 January 2026

**Available online:** 31 January 2026

#### Keywords:

botanical insecticide, *Vitex trifolia*, *Sitophilus oryzae*, pest, rice, leaf powder, mortality

*Sitophilus oryzae* (Coleoptera: Curculionidae) is a primary pest of stored rice that causes grain perforation, breakage, and powdering. This study evaluated the effectiveness of *Vitex trifolia* leaf powder as a botanical insecticide against *S. oryzae* under laboratory conditions. The experiment was conducted at the Laboratory of Plant Protection, Faculty of Agriculture, Syiah Kuala University, from December 2024 to March 2025, using a completely randomized design (CRD) with six treatments and four replications. The treatments consisted of 0 (control), 5, 10, 15, 20, and 25 g of *V. trifolia* leaf powder per 100 g of rice. The observed parameters included feeding deterrence caused by *V. trifolia* application, adult mortality, F1 adult emergence time, percentage inhibition of F1 adults' reproduction, percentage of rice weight loss, and the sex ratio of *S. oryzae*. The results demonstrated a clear dose-dependent response across all measured parameters. Feeding deterrence increased progressively from 44.50% at the 5 g dose to 54.50% at the 25 g dose. Similarly, adult mortality increased from 32.50% at the lowest dose to 82.50% at the highest dose, indicating that the insecticidal efficacy of *V. trifolia* leaf powder is positively correlated with application rate. F1 adults' emergence was delayed (42.32 days), and progeny numbers were reduced (36.7 individuals) at the 25 g dose. This treatment also resulted in the highest reproductive inhibition (79.58%) and the lowest rice weight loss (3.05%). The sex ratio was male-biased at all doses but decreased with increasing concentrations. Overall, *V. trifolia* leaf powder demonstrated significant potential as an environmentally safe botanical insecticide against *S. oryzae*. The 25 g/100 g dose provided the most comprehensive protection for stored rice, supporting the use of this locally available plant material as a viable alternative to synthetic pesticides in sustainable stored-grain pest management systems.

## 1. INTRODUCTION

Rice (*Oryza sativa*) is the primary staple food for most of the Indonesian population, and its demand continues to increase in line with population growth [1]. This increasing demand poses a threat to national food security [2]. One of the major pests of stored rice is *Sitophilus oryzae* (Coleoptera: Curculionidae), which causes significant post-harvest damage by boring into rice grains, resulting in breakage, powdering, and overall quality deterioration [3]. Therefore, effective control of *S. oryzae* is essential to minimize both quantitative and qualitative losses during storage [4].

Currently, the control of *Sitophilus oryzae* relies primarily on synthetic insecticides, which have adverse effects on human health, non-target organisms, and the environment. In addition, fumigation with phosphine and methyl bromide, commonly used in Indonesian rice storage facilities, may lead to residue accumulation in stored rice and the development of pest resistance [4]. Consequently, there is an urgent need for environmentally friendly alternatives, such as botanical insecticides [5].

Botanical insecticides have attracted increasing attention

due to their biodegradability, lower mammalian toxicity, and multiple modes of action [6, 7]. Plant-derived materials contain diverse secondary metabolites, including terpenoids, sesquiterpenes, alkaloids, flavonoids, glycosides, and phenolic compounds, which may exert insecticidal, repellent, antifeedant, and growth-regulating effects [8, 9]. Unlike single-active synthetic insecticides, botanical products may act through multiple physiological pathways, thereby potentially reducing the likelihood of resistance development [10]. Bioactive compounds in *V. trifolia* (e.g., casticin, artemetin, and vitricin) and their mechanisms of action have been investigated, including reported acetylcholinesterase (AChE) inhibition [6, 8].

*Vitex trifolia* L., traditionally used by local farmers in Aceh to repel storage pests by sprinkling dried leaves in rice warehouses, has shown potential as a botanical insecticide [11]. The leaves of *V. trifolia* contain various bioactive compounds, including sesquiterpenes, terpenoids, alkaloids, glycosides, and flavonoids. These compounds are known to inhibit insect growth, disrupt nervous system function, and exhibit repellent activity [11]. Previous studies reported that *V. trifolia* leaf extract at a concentration of 20% repelled

*Sitophilus oryzae* by 56.7% after 120 h, whereas a 1% concentration resulted in only 3.3% repellency after 24 h [12]. In powder form, 10 g/100 g rice reportedly repelled adults and caused adult mortality within 1 h of application, suggesting interference with the insect's respiratory and nervous systems.

Given these promising preliminary findings, *Vitex trifolia* leaf powder represents a viable botanical insecticide candidate for sustainable pest management in stored rice systems. However, while liquid extracts of *V. trifolia* have shown repellent activity against *S. oryzae* [12], the efficacy of its powdered leaf form—a simpler, more accessible, and more practical formulation for smallholder farmers—has not been comprehensively evaluated. Specifically, the dose-dependent effects of *V. trifolia* leaf powder on multiple biological endpoints, including adult mortality, F1 progeny development and emergence, reproductive inhibition, sex ratio dynamics, and grain weight loss, remain largely uncharacterized. Addressing this gap is essential for developing evidence-based recommendations for the use of botanical insecticides as alternatives to synthetic chemicals in rice storage facilities. This study aimed to (1) determine the dose-dependent repellent and lethal effects of *V. trifolia* leaf powder against adult *S. oryzae*; (2) assess its impact on F1 progeny development, emergence, and sex ratio; and (3) quantify the resulting protection of rice grains from weight loss.

## 2. MATERIAL AND METHODS

### 2.1 Research tools and materials

The equipment used in this study included an analytical balance, a laboratory blender, scissors, forceps, a stereo binocular microscope, a digital camera, a 30-mesh sieve, and measuring spoons.

The materials comprised milled rice obtained from the Meutuah Baro rice mill, label paper, plastic containers (thinwall; 3000 and 450 mL), brushes, Petri dishes, spatulas, 30 mL film bottles, plastic sheets, rubber bands, 70% ethanol, gauze, tea sachets (8 × 10 cm), laboratory gloves, and stationery. Adult *Sitophilus oryzae* were collected from the Meutuah Baro rice mill in Tungkop Village, Aceh Besar Regency. The collected insects were subsequently reared in the laboratory on untreated milled rice (250 g per container) for a minimum of two generations under controlled conditions (temperature: 27 ± 2°C; relative humidity: 65 ± 5%; photoperiod: 12:12 h light:dark) to establish a homogeneous laboratory colony. Only unsexed, newly emerged adults (0–7 days post-eclosion), identified by their lighter body coloration, were selected for use as test insects in all bioassays. Prior to each experiment, test insects were starved for 5 h to standardize hunger levels. Leaves of *Vitex trifolia* used for powder preparation were collected from rice fields in Neusok Village, Darul Kamal District, Aceh Besar Regency.

Freshly collected *V. trifolia* leaves were thoroughly washed with tap water to remove surface contaminants and rinsed with distilled water. The washed leaves were air-dried under shade at ambient temperature (28–30°C) for four days until brittle and easily crumbled by hand. The dried leaves were then pulverized using a laboratory blender and sieved through a 30-mesh sieve (aperture size: 0.595 mm) to obtain a uniform fine powder. The resulting powder was stored in airtight plastic containers at room temperature (27 ± 2°C) and used within two weeks of preparation. For each experimental unit, a

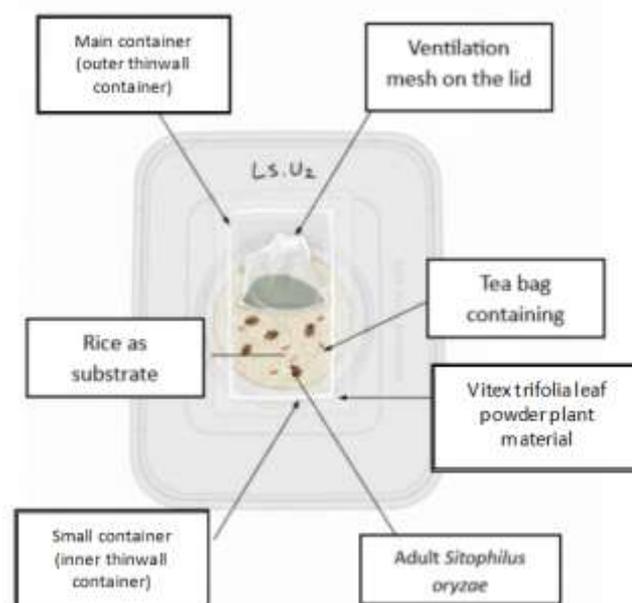
predetermined amount of powder (5, 10, 15, 20, or 25 g) was individually weighed using an analytical balance and enclosed in a tea sachet (8 × 10 cm) prior to application.

### 2.2 Research procedure

This study used a nonfactorial completely randomized design (CRD) with 6 treatments and 4 replications. Each treatment was replicated four times, resulting in a total of 24 experimental units. The arrangement of the *V. trifolia* leaf powder treatments is presented in the following Table 1 and Figure 1.

**Table 1.** Arrangement of treatment doses of *V. trifolia* leaf powder

No.	Treatment	Dose of <i>V. trifolia</i> Leaf Powder / 100 g Rice
1	L0	0 g (Control)
2	L1	5 g
3	L2	10 g
4	L3	15 g
5	L4	20 g
6	L5	25 g



**Figure 1.** The schematic diagram of the experimental setup

#### 2.2.1 Percentage of repellent power against adults *Sitophilus oryzae*

Repellent activity was evaluated using a confined-space avoidance assay. The experimental arena consisted of a cylindrical plastic container (thinwall; diameter: 12 cm, height: 8 cm, capacity: 450 mL) with a perforated lid sealed with gauze to allow air exchange while preventing insect escape. For the repellency test, the container was divided into two conceptual zones: a treatment zone, where one tea sachet containing the designated dose of *V. trifolia* leaf powder was placed at one end of the container base, and an opposite avoidance zone at the other end. No rice was included in this phase to isolate the olfactory repellent effect from feeding behavior. Five pairs of adult *S. oryzae* (10 individuals total) were introduced at the center of the container. The container was then sealed. After 2 hours of exposure, the number of

adults located in the avoidance zone (i.e., at the opposite half of the container from the sachet) was recorded. Adults found actively climbing the container walls away from the sachet were also counted as repelled. This repellency test was conducted independently and prior to the mortality bioassay. Repellency was calculated using the formula described by [13]:

$$DR = \frac{JA - JP}{JA + JP} \times 100\% \quad (1)$$

where,

$DR$  = Repellent Power,  $JA$  = Initial number of adults,  $JP$  = Number of adults around the treatment object.

### 2.2.2 Adult mortality of *Sitophilus oryzae*

Following the repellency assessment, 100 g of untreated milled rice was added to the same thinwall container (cylindrical; diameter: 12 cm, height: 8 cm, capacity: 450 mL) containing the tea sachet of *V. trifolia* leaf powder and the five pairs of *S. oryzae* adults. The sachet was positioned centrally within the rice to ensure uniform distribution of volatile compounds. Each container was sealed with a perforated lid covered with gauze to permit ventilation while preventing insect escape, and maintained under controlled laboratory conditions (27 +/- 2°C; 65 +/- 5% RH; 12:12 h L:D). Mortality was recorded daily from 1 to 15 days after infestation (DAI). An insect was considered dead when it showed no movement upon gentle prodding with a fine brush. No mortality was observed in the control group (0 g treatment) throughout the 15-day observation period; therefore, Abbott's correction was not applied, and the observed mortality values were used directly. Mortality was calculated using the formula described by Abbott [14].

$$P_0 = \frac{r}{n} \times 100\% \quad (2)$$

where,

$P_0$  = Adult mortality rate,  $r$  = Number of adults that died,  $n$  = Total number of adults.

### 2.2.3 Time to first F1 adult emergence of *Sitophilus oryzae*

Any remaining adult insects in the experimental units were removed along with the bags containing *V. Trifolia* leaf powder after 15 days of exposure. The duration of F1 emergence was then monitored from 25 days after infestation in each experiment until the first F1 adult emerged.

### 2.2.4 Number of F1 adults emerged

Observations on the number of F1 adults of *Sitophilus oryzae* were conducted 60 days after infestation (DAI). Each experimental unit (thinwall container) was opened, and the rice, along with the insects, was emptied onto a tray. All emerging adults were collected and preserved in a 30 mL film bottle containing 15 mL of 70% ethanol. adults remaining within the rice grains were subsequently removed and combined with the initial collection.

### 2.2.5 Percentage of reproductive inhibition in F1

Observations on the number of F1 adults of *Sitophilus oryzae* were conducted 60 days after infestation (DAI). The percentage inhibition of F1 reproductive capacity was determined at 60 DAI to evaluate the effect of *Vitex trifolia*

leaf powder on F1 reproduction. The inhibition percentage was calculated using the formula described by research [15].

$$IR = \frac{Cn - Tn}{Cn} \times 100\% \quad (3)$$

where,

$IR$  = Inhibition of reproductive power,  $Cn$  = Number of insects appearing on thinwall without treatment (control),  $Tn$  = Number of insects appearing on thinwall given treatment.

### 2.2.6 Percentage grain weight loss

The percentage of rice weight loss was observed 60 days after application. Weight loss was calculated using a digital scale by weighing the damaged rice after treatment. The damaged rice weight data was then used to calculate the percentage of rice damage using the following formula.

$$\text{Weight Loss (\%)} = \frac{\text{Initial weight (g)} - \text{Damaged weight (g)}}{\text{Initial weight}} \times 100\% \quad (4)$$

### 2.2.7 Sex ratio of F1 adults of *Sitophilus oryzae*

Sex ratio observations were conducted simultaneously with the number of F1 adults at 60 days after application. All F1 adults were placed in film bottles containing 70% alcohol and observed using a stereo microscope to determine the ratio of male and female *S. oryzae* adults.

The sex ratio was calculated using the following formula:

$$\text{Sex Ratio} = \frac{\text{Number of male adults}}{\text{Number of female adults}} \quad (5)$$

## 2.3 Statistical analysis

All data were analyzed using a one-way analysis of variance (ANOVA) appropriate for a CRD. Prior to analysis, data were assessed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Percentage data, including repellency, mortality, and reproductive inhibition rates, were transformed using the arcsine square root transformation ( $\arcsin \sqrt{x}$ ) to meet the assumptions of normality and homoscedasticity. Count data, including the number of F1 adults emerged, were transformed using the square root transformation ( $\sqrt{x + 0.5}$ ). When the ANOVA indicated significant treatment effects ( $P < 0.05$ ), means were separated using the Least Significant Difference (LSD) test at the 0.05 significance level [16]. Results are presented as untransformed means for clarity, while statistical significance was determined based on the transformed data.

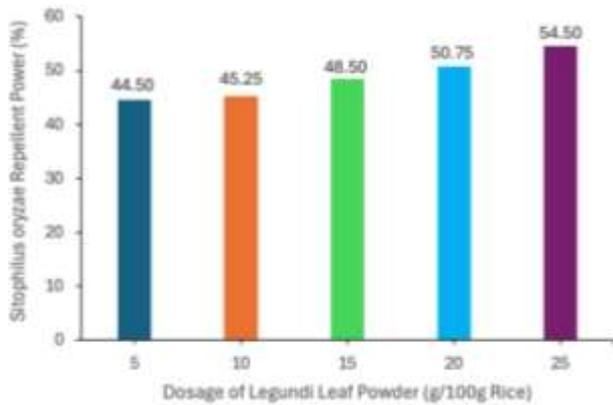
## 3. RESULTS AND DISCUSSION

### 3.1 Percentage of repellent power of *Sitophilus oryzae* adults

Figure 2 shows that the mean repellency of adult *Sitophilus oryzae* following application of *Vitex trifolia* leaf powder differed significantly among treatments ( $F = 21.85$ ;  $P < 0.01$ ). The highest repellency was recorded at the 25 g dose (54.50%), whereas the lowest was observed at the 5 g dose (44.50%).

The repellent activity of *Vitex trifolia* leaf powder is

associated with the presence of terpenoid compounds, particularly essential oils with fumigant properties. These compounds are reported to interfere with the nervous system of *Sitophilus oryzae* and emit odors that are unfavorable to insects, resulting in reduced activity and avoidance behavior. Increasing doses of *V. trifolia* leaf powder correspond to higher concentrations of bioactive compounds, thereby enhancing its effectiveness as a botanical insecticide.



**Figure 2.** The insect repellent power of *Sitophilus oryzae* was observed in 2 days after application (DAA)

Terpenoids present in *V. trifolia* leaves have been reported to exhibit toxic and repellent effects against various insect pests [12, 17]. Essential oils primarily exert their repellent action through the respiratory system, whereby inhaled volatile compounds diffuse throughout the insect body and disrupt neural function, potentially leading to mortality [12, 18]. Repellency is attributed to volatile terpenoids (e.g., sesquiterpenes in essential oils) acting as fumigants through olfactory disruption and interference with octopaminergic

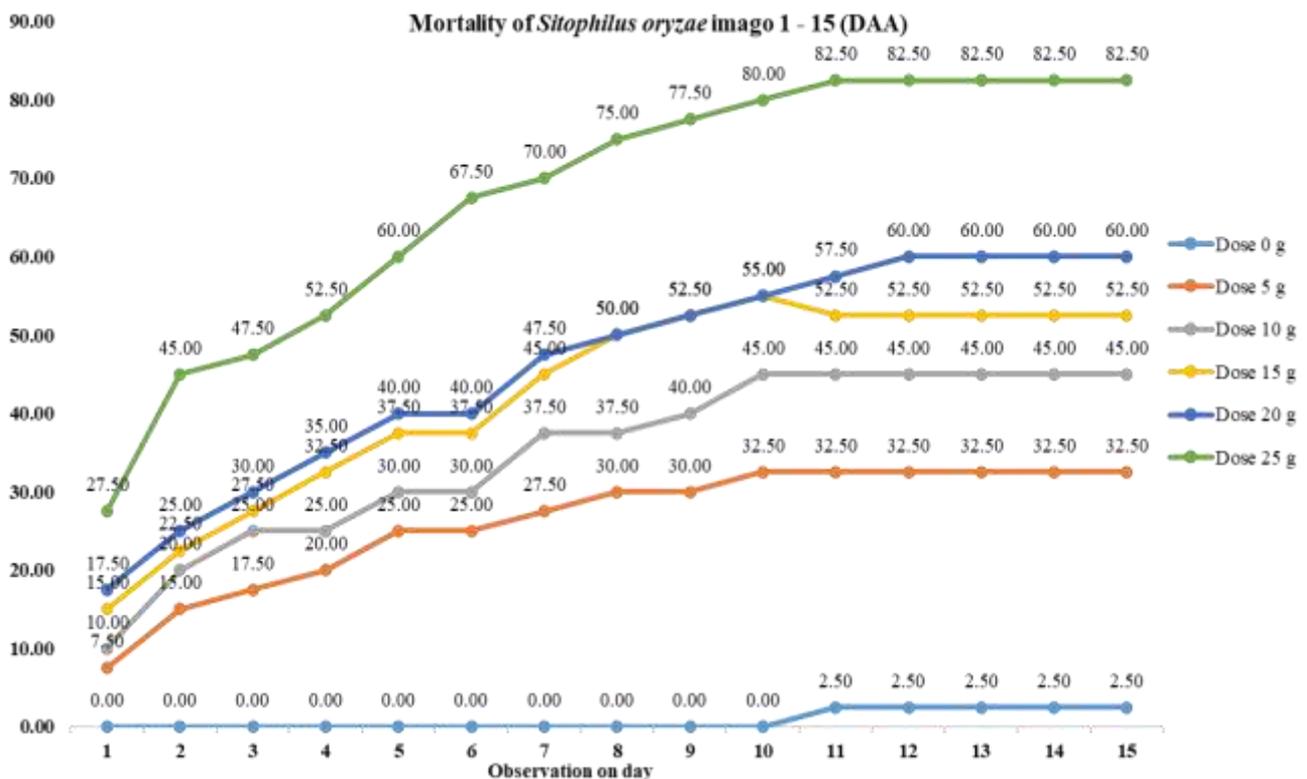
receptors [19].

### 3.2 Mortality of *Sitophilus oryzae* adults

The insecticidal activity of *Vitex trifolia* leaf powder against *S. oryzae* adults was dose- and time-dependent (Figure 3). Mortality across all treatment groups increased progressively from day 1 to day 9 after application (DAA). The highest application rate of 25 g exhibited the most pronounced effect, with mortality rising sharply from 27.50% on day 1 to 77.50% by day 9. This trend continued, reaching a peak of 82.50% between 10 and 11 DAA, after which it plateaued until the end of the observation period on day 15. The 20 g dose also showed substantial efficacy, achieving 52.50% mortality by day 9.

In contrast, lower doses resulted in considerably slower and lower mortality. By day 9, the 10 g and 5 g doses had only induced 40.00% and 30.00% mortality, respectively. Mortality at the 5 g dose remained consistently low, stabilizing at approximately 32.50% from day 10 onwards. The 15 g dose showed an intermediate effect, reaching 52.50% mortality by the end of the trial.

This response is likely influenced by the essential oil content of *V. trifolia* leaf powder, which functions as a repellent through the emission of strong odors that drive *Sitophilus oryzae* away from food sources. In addition, volatile compounds such as terpenoids and alkaloids act as respiratory toxins that can enter the insect body through the spiracles, disrupt nervous system function, and cause physiological tissue damage, ultimately leading to mortality. This finding is consistent with that of researcher [20], who reported that alkaloids and tannins act as antifeedants by reducing larval feeding activity, thereby decreasing nutrient intake. Such nutritional stress may be further exacerbated by saponins, which interfere with metabolic processes and induce fluid loss.



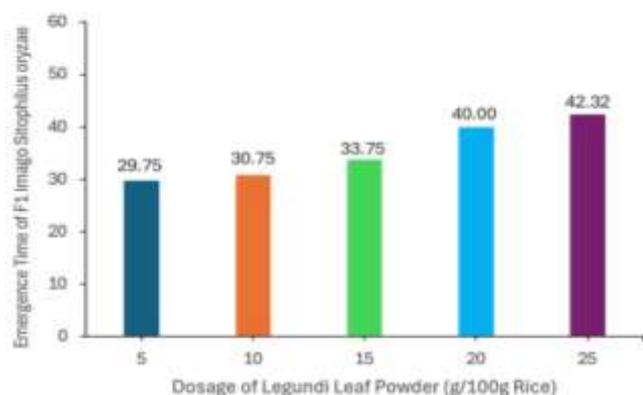
**Figure 3.** Mortality of *Sitophilus oryzae* adults following the application of *Vitex trifolia* leaf powder was observed at 1–15 days after application (DAA)

According to Ainun et al. [12], higher application doses result in stronger repellent effects on rice weevils, and prolonged exposure to essential oils increases insect migration away from food sources. Similarly, Utomo et al. [4] reported that increasing the dosage of botanical insecticides is directly proportional to their effects on target organisms, as higher amounts of active compounds enter the insect body. Comparable results have been observed with *V. trifolia* leaf extract, which caused increased mortality of *Spodoptera litura* larvae at 36 h after application.

It is noteworthy that mortality at the earlier observation points (5, 7, and 9 DAA) did not differ significantly among treatments ( $p > 0.05$ ), despite clear numerical trends. This lack of statistical significance may be attributed to several factors: (1) the inherent high variability among replications in botanical insecticide bioassays, as the bioactive compound concentrations in plant-derived preparations are less uniform compared to synthetic formulations; (2) the volatile nature of essential oils, whose active compounds may evaporate at different rates across replications depending on micro-environmental conditions within each container; and (3) the relatively small sample size (10 adults per experimental unit), which may limit the statistical power to detect moderate treatment effects. However, the cumulative mortality data at days 10-15 revealed more pronounced dose-dependent differences, suggesting that the insecticidal effect of *V. trifolia* leaf powder requires sustained exposure for full expression. This time-dependent pattern is consistent with the mode of action of essential oil-based insecticides, which act primarily through chronic respiratory exposure and gradual disruption of neural and metabolic functions rather than acute contact toxicity. Furthermore, adult mortality is linked to alkaloids (e.g., vitricin) and saponins, which cause acetylcholinesterase (AChE) inhibition and cell membrane disruption, respectively [21].

### 3.3 Time to first F1 adult emergence of *Sitophilus oryzae*

As shown in Figure 4, the mean F1 adult emergence time of *Sitophilus oryzae* differed significantly among *Vitex trifolia* leaf powder treatments. Increasing application rates significantly delayed development to the adult stage. Developmental delay in F1 is associated with flavonoid glycosides (artemetin, casticin) that interfere with juvenile hormone and ecdysone-mediated developmental pathways [22]. The longest emergence time was recorded at the 25 g dose (42.32 days), whereas the shortest was observed at the 5 g dose (29.75 days).



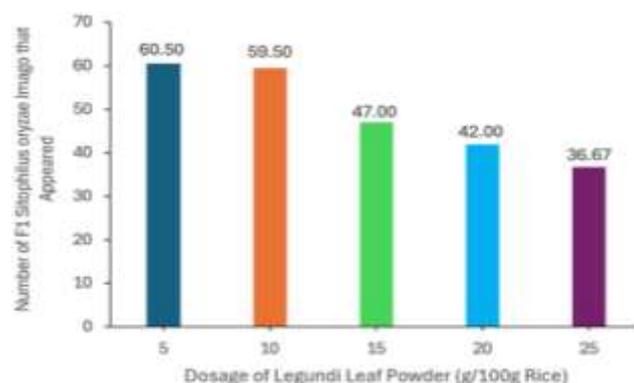
**Figure 4.** The emergence time of F1 adults *Sitophilus oryzae* at 60 days after application (DAA) observations

Differences in adult emergence time indicate that increasing doses of *Vitex trifolia* leaf powder prolong the life cycle of *Sitophilus oryzae*. This effect may be associated with the activity of bioactive compounds, such as essential oils, flavonoids, and alkaloids, which are known to inhibit insect development within rice grains. Previous studies have reported that delayed adult emergence of *Sitophilus zeamais* is influenced by essential oil compounds and alkaloids, including dillapiole, myristicin, Z-carpacin, piperamide, and constituents of clove flower essential oil (eugenol, caryophyllene,  $\alpha$ -caryophyllene, and eugenol acetate), which disrupt insect reproductive systems [23]. Prolongation of the egg, larval, and pupal stages consequently increases the time required for adult emergence. This finding is consistent with Hikal et al. [10], who reported that essential oils exhibit repellent, growth-inhibitory, ovicidal, and oviposition-suppressing effects, thereby reducing the population growth rate of *Sitophilus* spp.

### 3.4 Number of F1 adults emerged

Based on Figure 5, the application of *V. trifolia* leaf powder resulted in a highly significant difference in the number of F1 adults *S. oryzae* emerging among treatments ( $F = 10.11$ ;  $p < 0.01$ ). The lowest number of F1 adults was recorded at a dose of 25 g, i.e., 36.67 individuals, while the highest number occurred at a dose of 5 g with an average of 60.50 individuals.

The variation in the number of F1 adults s emerging between treatments is thought to be related to the activity of chemical compounds in the powdered leaves of the *V. trifolia*. The aroma of these compounds affects the physiological activity of *S. oryzae*, including nervous system disruption, decreased feeding activity, and reduced copulation frequency.



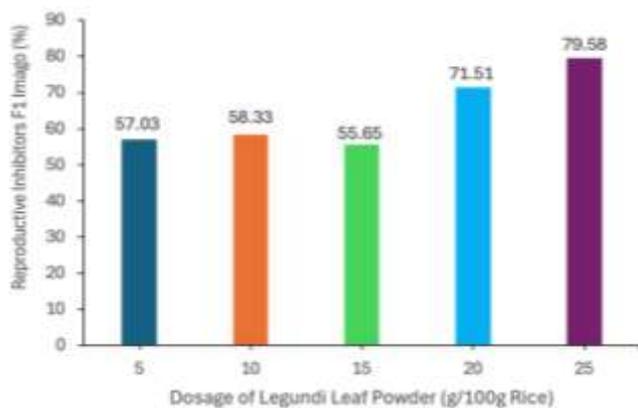
**Figure 5.** Number of F1 adults of *S. oryzae* appearing at 60 days after application (DAA) observation

Ripoll-Aristizábal et al. [24] reported that the application of sourrop leaf flour disrupted oviposition and reduced the hatchability of *Callosobruchus analis* beetle eggs on mung bean seeds. *Azadirachtin*, a terpenoid derivative with a pungent aroma, is known to have repellent and antioviposition activities, reduce fecundity, and inhibit insect growth and development [25]. also stated that antifeedant compounds can inhibit the copulation of female *S. oryzae* adults and reduce feeding activity, so that increasing the dose has implications for reducing the number of F1 adults that emerge. In addition, *flavonoids* and *tannins* have been reported to interfere with feed digestion by reducing the activity of *protease* and *amylase enzymes*, which in turn inhibits insect growth and development.

### 3.5 Percentage inhibition of F1 adult emergence

As shown in Figure 6, *Vitex trifolia* leaf powder significantly affected the inhibition rate (IR) of F1 adult emergence of *Sitophilus oryzae* ( $F = 12.15$ ;  $p < 0.01$ ). The highest reproductive inhibition was recorded at the 25 g dose (79.58%), whereas the lowest inhibition occurred at the 15 g dose (55.65%).

This variation is likely associated with differences in the concentration of bioactive compounds present in *V. trifolia* leaf powder, particularly terpenoids, saponins, and alkaloids, which have been reported to suppress insect reproductive activity. The inhibition of reproduction is likely attributed to the combined action of terpenoids and phenolic compounds, which may cause oviposition deterrence and ovicidal effects [10].



**Figure 6.** Average percentage inhibition of F1 adult emergence

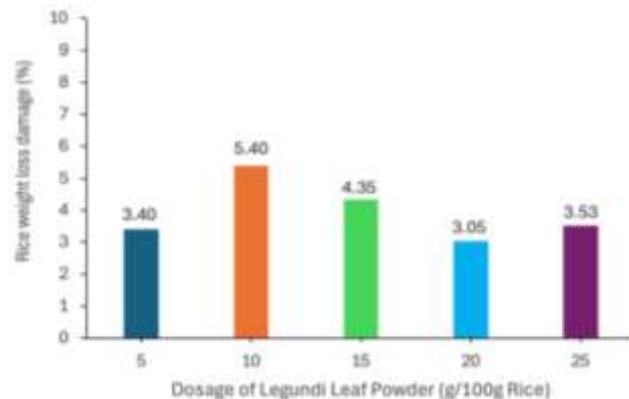
This finding is consistent with the researchers [26, 27], who reported that low F1 adult emergence is influenced by the mortality of infesting adults. The applied materials are known to contain volatile compounds, including phenols, flavonoids, and saponins, which exhibit toxic effects against insect pests. According to researchers [28], inhibition of adult emergence may result from volatile compounds derived from botanical insecticides that induce mortality in the egg and larval stages of *Sitophilus oryzae*.

Essential oils contribute to the repellent activity against rice weevils; moreover, Abdellaoui et al. [29] reported that essential oils possess both repellent and toxic properties. The insecticidal activity of essential oils is dose-dependent, whereby sublethal concentrations primarily induce repellent responses, while higher doses reduce egg viability and oviposition success. In addition, flavonoids exhibit a wide range of biological activities; although commonly recognized for their antioxidant and anti-inflammatory properties [30], flavonoids have also been reported to exert toxic effects on insects [31]. Phenolic compounds within flavonoids contribute less to repellent activity due to their low volatility; however, they may negatively affect insect fitness by reducing fertility and lifespan.

### 3.6 Percentage grain weight loss

Based on Figure 7, the average percentage of rice weight loss caused by *Sitophilus oryzae* infestation did not differ significantly among treatments, although numerical variations were observed. The highest rice weight loss occurred at the 10

g dose (5.40%), followed by the 15 g dose (4.35%). The lowest rice weight loss was recorded at the 20 g dose (3.05%), closely followed by the 5 g dose (3.40%) and the 25 g dose (3.53%). The non-linear pattern across treatment doses, where the 5 g dose showed lower weight loss than higher doses such as 10 g and 15 g, may be attributable to inherent variability in individual insect feeding behavior and oviposition patterns among replications.



**Figure 7.** Average percentage of damage due to weight loss in rice

Rice weight loss was positively correlated with the population density of *S. oryzae* adults in each treatment. Higher adult populations resulted in greater levels of grain damage and weight loss, while treatments with lower adult populations exhibited reduced rice weight loss. This relationship indicates that the extent of rice damage is strongly influenced by the abundance of *S. oryzae* adults present during the storage period.

*Sitophilus* spp. utilize rice grains both as a food source and as an oviposition site by boring into the kernels to deposit eggs. Elimam et al. [17] reported that a reduction in pest population density is associated with a corresponding decrease in the percentage of damaged grains, which directly leads to lower rice weight loss. Reduced grain damage results in minimized quantitative losses because fewer kernels are physically destroyed during infestation.

Hidayati et al. [32] reported that rice grains with high nutrient content, relatively high moisture levels, and a softer texture provide favorable conditions for the growth and development of *Sitophilus oryzae*. Such characteristics create an optimal environment for feeding activity, oviposition, and successful completion of the insect's life cycle within the grain. Consequently, food substrate suitability plays a crucial role in shaping population dynamics, as *S. oryzae* preferentially selects media with adequate nutritional quality to support survival and reproduction. Although *S. oryzae* requires nutrients throughout its life cycle, rice with high amylose content alone, in the absence of sufficient nutritional support, is insufficient to sustain long-term population growth.

### 3.7 Sex ratio of F1 adults of Sitophilus oryzae

The results of observations on the sex ratio due to the application of the *V. trifolia* leaf powder treatment can be seen in Table 2 below.

Based on Table 2, the aroma emitted by *Vitex trifolia* leaf powder appeared to influence the sex ratio of adult *Sitophilus oryzae*. At a dose of 10 g, the numbers of male and female

adults were 231 and 41, respectively. In contrast, application of 25 g resulted in a markedly lower adult emergence, with 84 males and 23 females recorded. This effect is likely associated with the activity of bioactive compounds present in *V. trifolia* leaf powder, which interfere with the reproductive capacity of *S. oryzae*. These compounds may reduce nutrient intake and disrupt physiological processes during development, leading to impaired regeneration and an imbalance in the F1 sex ratio, characterized by a higher proportion of males than females.

**Table 2.** Sex ratio of *S. oryzae* adults due to the application of *V. trifolia* leaf powder

Treatment	Total Individuals	Male	Female	Sex Ratio
Control	581	452	129	3.50:1
5 g	196	160	36	4.44:1
10 g	272	231	41	5.63:1
15 g	263	231	32	7.22:1
20 g	198	164	34	4.82:1
25 g	110	84	23	3.65:1
Total	1620	1322	295	-

Hendriwal and Melinda [33] reported that limited food resources can influence the sex ratio of *Sitophilus zeamais* adults, with suboptimal nutritional conditions tending to produce a higher proportion of males than females. Conversely, under conditions of optimal nutrient availability, the male-to-female ratio tends to be more balanced [34]. This pattern is further supported by researcher [35], who demonstrated that increased food availability is positively correlated with a higher number of female adults, whereas nutritional constraints result in male-biased populations.

From an ecological and pest management perspective, the observed male-biased sex ratio at higher doses of *V. trifolia* leaf powder has important implications for long-term population suppression of *S. oryzae* in storage. A reduction in the proportion of females directly limits the reproductive potential of the population, as fewer females are available for oviposition in subsequent generations. Combined with the observed reduction in total F1 progeny numbers and delayed adult emergence, these effects suggest that *V. trifolia* leaf powder exerts multi-level suppressive pressure on *S. oryzae* populations not only through direct adult mortality, but also through disruption of reproductive output and population sex structure. In practical stored-grain management, this means that even sublethal doses that do not achieve high acute mortality may still provide meaningful long-term protection by progressively reducing the reproductive capacity and growth rate of pest populations across successive storage cycles.

### 3.8 Comparative efficacy with other botanical powders

Although LC<sub>50</sub> and LC<sub>90</sub> values were not calculated in the present study due to the design using fixed application rates in a rice matrix, the mortality level achieved at 25 g/100 g rice (82.50% at day 10–15) indicates relatively strong insecticidal activity compared with several plant powders previously reported against *S. oryzae*.

For example, *Annona squamosa* (Annona) leaf powder has been reported to cause mortality levels of weevils of about 98.3% after the 8<sup>th</sup> week [36]. Another study shows that the extract of *Cymbopogon citratus* root and leaves at 200 ppm cause 100% mortality of weevils after 24 h exposure [37]. The mortality levels of weevils observed in this study (82.50%

after 11 days) are also comparatively high that typically reported as moderate suppression of F<sub>1</sub> emergence [38].

Importantly, the present study used crude leaf powder rather than solvent extracts or purified essential oils. Considering that crude botanical materials generally exhibit lower bioavailability than concentrated extracts, the mortality and reproductive suppression levels observed here suggest promising relative efficacy. Future studies incorporating probit analysis to determine LC<sub>50</sub> and LC<sub>90</sub> values would further strengthen comparative evaluation and allow standardized toxicity assessment.

## 4. CONCLUSIONS

Based on this research data, it can be concluded that:

1. The application of 25 g of *Vitex trifolia* leaf powder exhibited the highest efficacy in suppressing the *Sitophilus oryzae* population, as evidenced by a repellency rate of 54.50% and adult mortality of 82.50%. This treatment also resulted in the longest F1 adult emergence time (42.32 days), the lowest number of F1 adults (36.67 individuals), and the highest reproductive inhibition (79.58%). In addition, rice weight loss at the 25 g dose was 3.53%, substantially lower than the control (9.33%), and the resulting sex ratio was male-biased, with 84 males compared to 23 females.
2. In contrast, the 5 g treatment exhibited the lowest effectiveness, as indicated by a repellency rate of 44.50% and adult mortality of 32.50%. This dose resulted in the shortest F1 adult emergence time (29.75 days) and the highest number of F1 adults (60.50 individuals). Reproductive inhibition was relatively low (55.65%), rice weight loss at the 5 g dose was 3.40%, and the resulting sex ratio was male-biased, with 160 males compared to 36 females.
3. Although the 25 g/100 g rice treatment exhibited the highest biological activity under laboratory conditions, this dose corresponds to a 25% (w/w) proportion, which may limit its direct application in commercial storage systems due to economic and operational constraints. This study constitutes a laboratory-based exploratory investigation aimed at demonstrating the effectiveness of *Vitex trifolia* leaf powder, which exhibited bioactivity and multi-level suppressive effects against *Sitophilus oryzae*, rather than serving as a technical recommendation for immediate implementation at a practical storage scale.

## ACKNOWLEDGMENT

The authors express their sincere gratitude and high appreciation to all parties who contributed to this research, particularly the Department of Plant Protection and the Plant Protection Laboratory, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh.

## REFERENCES

- [1] Artia, J.I., Mutiara, D., Novianti, D. (2022). Mortality test of rice weevil (*Sitophilus oryzae*) using biological

- control with *Beauveria bassiana*. *Indobiosains Journal*, 4(1): 9-14. <https://doi.org/10.31851/indobiosains.v4i1.6205>
- [2] Herlina, N., Wahyuni, H., Winahyu, F.P., Anwar, K., Heliana. (2024). The movement to maintain food security through technological advancements and local diversification: Ministry of Agriculture Republic of Indonesia. *Journal of Natural Resources and Environmental Management*, 14(3): 555-564. <https://doi.org/10.29244/jpsl.14.3.555>
- [3] Kadi Kadi, H.A., Ibrahim, A.M., Pendleton, B.B., Aboubacar, K. (2025). Efficacy of selected botanical powders to control maize weevil, *Sitophilus zeamais* Motschulsky in stored sorghum grain. *Journal of Agricultural Chemistry and Environment*, 14(1): 23-36. <https://doi.org/10.4236/jacen.2025.141002>
- [4] Utomo, A.W., Susilaningih, N., Armalina, D. (2015). Acute toxicity test of soursop leaves (*Annona muricata*) on liver and kidney of Switzerland mice. *Sains Medika: Jurnal Kedokteran dan Kesehatan*, 6(2): 48-51. <https://doi.org/10.30659/sainsmed.v6i2.600>
- [5] Wahjono, T.E., Suhatman, A., Wihermanto, W., Hadiyanto, H. (2024). Neem (*Azadirachta indica* A. Juss; Meliaceae) as the source for plant-based pesticides as an effective and sustainable biocontrol alternative. *Journal of Bioresources and Environmental Sciences*, 3(3): 128-141. <https://doi.org/10.61435/jbes.2024.19928>
- [6] Acheuk, F., Basiouni, S., Shehata, A.A., Dick, K., et al. (2022). Status and prospects of botanical biopesticides in Europe and mediterranean countries. *Biomolecules*, 12(2): 311. <https://doi.org/10.3390/biom12020311>
- [7] Chaudhary, S., Yadav, S.K., Verma, P., Sagar, S., Lal, M. (2024). Botanical insecticides for crop protection: Major classes and possible mechanisms of action. In *Insecticides in Pest Control - Impact, Challenges and Strategies*. <https://doi.org/10.5772/intechopen.1006743>
- [8] Karakoti, H., Bargali, P., Kumar, R., Prakash, O., Kumar, S., Garzoli, S., Rawat, D.S., Maslowiecka, J., Isidorov, V.A. (2025). Comparison of chemical composition, nematocidal activity and acetylcholinesterase inhibition with in silico mechanistic insights of *Hedychium flavescens* essential oils and extracts from aerial parts and rhizomes. *Biochemical Systematics and Ecology*, 121: 104987. <https://doi.org/10.1016/j.bse.2025.104987>
- [9] Pang, Y.P., Brimijoin, S., Ragsdale, D.W., Zhu, K.Y., Suranyi, R. (2012). Novel and viable acetylcholinesterase target site for developing effective and environmentally safe insecticides. *Current Drug Targets*, 13(4): 471-482. <https://doi.org/10.2174/138945012799499703>
- [10] Hikal, W.M., Baeshen, R.S., Said-Al Ahl, H.A.H. (2017). Botanical insecticide as simple extractives for pest control. *Cogent Biology*, 3(1): 1404274. <https://doi.org/10.1080/23312025.2017.1404274>
- [11] Barreto, I.C., de Almeida, A.S., Sena Filho, J.G. (2021). Taxonomic insights and its type cyclization correlation of volatile sesquiterpenes in *Vitex* species and potential source insecticidal compounds: A review. *Molecules*, 26(21): 6405. <https://doi.org/10.3390/molecules26216405>
- [12] Ainun, A.D.A., Sjam, S., Sulaeha, S., Fitriyanti, N.A. (2025). Evaluating the repellent efficacy of *Vitex trifolia* leaf powder botanical formulation against oriental fruit flies (*Bactrocera dorsalis*). *HAYATI Journal of Biosciences*, 32(5): 1251-1259. <https://doi.org/10.4308/hjb.32.5.1251-1259>
- [13] Inyang, U.E., Emosairue, S.O. (2005). Laboratory evaluation of repellent and antifeedant properties of aqueous extracts from 13 plant species against the banana weevil *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). *Tropical and Subtropical Agroecosystems*, 5: 33-44. <https://www.redalyc.org/pdf/939/93950104.pdf>
- [14] Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18(2): 265-267. <https://doi.org/10.1093/jee/18.2.265a>
- [15] Tegegne, B. (2017). Combination effect of different insecticide plants against *Acanthoscelides obtectus* (Coleoptera: Bruchidea): Storage pests of common bean (*Phaseolus vulgaris*). *Journal of Agricultural Science and Food Research*, 8(4): 1-7.
- [16] Gomez, K.A., Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research*. New York: Wiley. <https://archive.org/details/statisticalproce00gome/page/n5/mode/2up>.
- [17] Elimam, A.M., Elmalik, K.H., Ali, F.S. (2009). Larvicidal, adult emergence inhibition, and oviposition deterrent effects of foliage extract from *Ricinus communis* L. against *Anopheles arabiensis* and *Culex quinquefasciatus* in Sudan. *Tropical Biomedicine*, 26(2): 130-139. <https://pubmed.ncbi.nlm.nih.gov/19901899/>.
- [18] Purwatiningsih, P., Mandasari, F.P., Fajariyah, S. (2019). Toxicity of n-hexane extract of sengon wood sawdust (*Albizia falcataria* L. Forberg) on mortality of coffee berry borer (*Hypothenemus hampei* Ferr.) (Coleoptera: Scolytidae). *Journal of Tropical Biology*, 3(1): 39-48. <https://doi.org/10.29080/biotropic.2019.3.1.39-48>
- [19] Kostyukovsky, M., Rafaeli, A., Gileadi, C., Demchenko, N., Shaaya, E. (2002). Activation of octopaminergic receptors by essential oil constituents isolated from aromatic plants: Possible mode of action against insect pests. *Pest Management Science*, 58(11): 1101-1106. <https://doi.org/10.1002/ps.548>
- [20] Rian, I. (2021). Application of leaf extract formulation (*Vitex trifolia* L.) and black soldier fly frass (*Hermetia illucens*) against several pests on melon plants. Undergraduate thesis, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. <https://repository.unhas.ac.id/id/eprint/19332/>.
- [21] Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51: 45-66. <https://doi.org/10.1146/annurev.ento.51.110104.151146>
- [22] Regnault-Roger, C., Vincent, C., Arnason, J.T. (2012). Essential oils in insect control: Low-risk products in a high-stakes world. *Annual Review of Entomology*, 57: 405-424. <https://doi.org/10.1146/annurev-ento-120710-100554>
- [23] de Carvalho Brito, R., de Moura Pádua, L.E., da Silva, L.R., Briozzo, M.E.O., et al. (2024). Use of essential oils and  $\alpha$ -pinene as insecticides against *Sitophilus zeamais* and their effects on maize seed germination. *Agronomy*, 14(10): 2282. <https://doi.org/10.3390/agronomy14102282>
- [24] Ripoll-Aristizábal, D.C., Patiño-Ladino, O.J., Prieto-Rodríguez, J.A. (2025). Essential oils and

- phenylpropanoids from *Piper*: Bioactivity and enzyme inhibition in *Sitophilus zeamais* and *Tribolium castaneum*. *Journal of Stored Products Research*, 114: 102714. <https://doi.org/10.1016/j.jspr.2025.102714>
- [25] Harinta, Y., Setyorini, A. (2018). The effect of the flour of soursop and seed (*Annona muricata*) against the mortality and reproduction of *Callosobruchus analis* F. at the soybean and the mung bean. In *Proceedings of the International Conference on Applied Science and Engineering (ICASE 2018)*, pp. 51-55. <https://doi.org/10.2991/icase-18.2018.14>
- [26] Senthil-Nathan, S. (2013). Physiological and biochemical effects of neem and other botanical insecticides on insects. *Frontiers in Physiology*, 4: 359. <https://doi.org/10.3389/fphys.2013.00359>
- [27] Widiyaningrum, P., Candrawati, D., Indriyanti, D.R., Priyono, B. (2019). Repellent activity of waste extract from two local medicinal plants against *Sitophilus oryzae*. *Biosaintifika: Journal of Biology & Biology Education*, 11(1): 62-67. <https://doi.org/10.15294/biosaintifika.v11i1.17651>
- [28] de Oliveira, A.P.S., Agra-Neto, A.C., Pontual, E.V., de Albuquerque Lima, T., et al. (2020). Evaluation of the insecticidal activity of *Moringa oleifera* seed extract against *Sitophilus zeamais*. *Journal of Stored Products Research*, 87: 101615. <https://doi.org/10.1016/j.jspr.2020.101615>
- [29] Abdellaoui, M., Bouhlali, E.D.T., Kasrati, A., El Rhaffari, L. (2017). The effect of domestication on seed yield, essential oil yield, and antioxidant activities of fennel seed (*Foeniculum vulgare* Mill.) grown in Moroccan oases. *Journal of the Association of Arab Universities for Basic and Applied Sciences*, 24: 107-114. <https://doi.org/10.1016/j.jaubas.2017.06.005>
- [30] Eesiah, S., Yu, J., Dingha, B., Amoah, B., Mikiashvili, N. (2022). Preliminary assessment of repellency and toxicity of essential oils against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) on stored organic corn grains. *Foods*, 11(18): 2907. <https://doi.org/10.3390/foods11182907>
- [31] Panche, A.N., Diwan, A.D., Chandra, S.R. (2016). Flavonoids: An overview. *Journal of Nutritional Science*, 5: e47. <https://doi.org/10.1017/jns.2016.41>
- [32] Hidayati, N.A., Listyawati, S., Setyawan, A.D. (2008). Chemical constituents and anti-inflammatory test of ethanolic extracts of *Lantana camara* L. on white male rats (*Rattus norvegicus* L.). *Biotechnology*, 5(1): 10-17. <https://doi.org/10.13057/biotek/c050102>
- [33] Hendriwal, H., Melinda, L. (2019). Population growth of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and characteristics of weight loss in rice. *Biospecies*, 10(1): 17-24. <https://doi.org/10.22437/biospecies.v10i1.3484>
- [34] Manueke, J., Pelealu, J. (2015). Attractiveness of *Sitophilus oryzae* to rice, shelled maize, peanuts, soybeans, and copra. *Eugenia*, 21(2): 70-79. <https://doi.org/10.35791/eug.21.2.2015.9706>
- [35] Manueke, J., Tulung, M., Mamahit, J.M.E. (2015). Biology of *Sitophilus oryzae* and *Sitophilus zeamais* (Coleoptera: Curculionidae) on rice and shelled maize. *Eugenia*, 21(1): 20-31. <https://doi.org/10.35791/eug.21.1.2015.11802>
- [36] Romoli, O., Serrato-Salas, J., Gapp, C., Epelboin, Y., Figueras Ivern, P., Barras, F., Gendrin, M. (2024). Nutritional sex-specificity on bacterial metabolites during mosquito (*Aedes aegypti*) development leads to adult sex-ratio distortion. *Communications Biology*, 7: 1603. <https://doi.org/10.1038/s42003-024-07319-7>
- [37] Zainalabidin, N., Sirozi, M.I., Elias, N.H. (2024). Bioinsecticidal activity of roots and leaves extract of *Cymbopogon citratus* on *Sitophilus oryzae*. *Advanced and Sustainable Technologies (ASET)*, 3(2): 123-132. <https://doi.org/10.58915/aset.v3i2.1271>
- [38] Shashikumar, C., Singha, S., Mitra, S. (2025). Assessment of protective ability of some selected leaf powders against rice weevil (*Sitophilus oryzae*) and their effect on sensory properties of two exotic rice varieties. *Journal of Natural Pesticide Research*, 14: 100142. <https://doi.org/10.1016/j.napere.2025.100142>

## NOMENCLATURE

DAA	days after application
F1	first filial generation
g	gram
CRD	completely randomized design
DAI	days after infestation