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Corresponding Author:

Name : Debby Dijola Moniharapon
Email: debbydijola10@gmail.com

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**Debby Dijola Moniharapon¹, Evelin Tuhumuri²,
Mechiavel Moniharapon³, Cindri Tasane⁴**

¹⁻⁴Department of Biology, Faculty of Science and Technology,
Universitas Pattimura, Ambon – Indonesia

Abstract

The maize weevil (*Sitophilus zeamais*) is a major postharvest pest that frequently damages stored grains, as evidenced by characteristic feeding perforations. The use of botanical insecticides represents an environmentally friendly alternative for pest management. One promising natural resource is lime leaf (*Citrus aurantifolia*). This study aimed to evaluate the effectiveness of lime leaf extract on the mortality of the maize weevil (*Sitophilus zeamais*). A laboratory-based experimental approach was employed using five dosage treatments (0 g as the control, 3 g, 8 g, 13 g, and 18 g), each replicated three times. Observations were conducted at 0, 12, 14, 16, and 18 hours after exposure. Data were analyzed using a two-way analysis of variance (two-way ANOVA) to simultaneously assess the effects of dosage and exposure time on pest mortality. The results indicated that lime leaf extract was effective as a botanical insecticide, with mortality rates increasing in proportion to both dosage and duration of exposure. The most effective treatment was 18 g, which resulted in the highest mortality rate of 98.34% after 18 hours. These findings suggest that lime leaf extract has strong potential for development as a botanical insecticide for sustainable and environmentally safe storage pest management.

INTRODUCTION

Maize (*Zea mays* L.) is one of the primary staple foods in Indonesia after rice (Saleula & Maryam, 2018). It represents a major carbohydrate source and contains substantial amounts of protein, vitamins A, B, and E, as well as essential minerals (Murningsih et al., 2019). Globally, maize ranks third after rice and wheat in terms of production and consumption. In Indonesia, maize plays a strategic role in the national economy, serving as food, animal feed, and raw material for biofuel and bioethanol industries (Amzeri, 2018).

According to reports issued by the Ministry of Agriculture and national media publications, Indonesia's corn production in 2025 is projected to reach 16.55 million tons, representing a 9.34% increase compared with the previous year (Badan Pusat Statistik, 2024; Ministry of Agriculture of the Republic of Indonesia, 2025). This increase underscores the critical role of corn as a strategic commodity in supporting national food security and the agribusiness sector, while simultaneously serving as a primary source of livelihood for millions of farmers (OECD & FAO, 2023).

Storage constitutes a critical final stage in postharvest management. However, during storage, agricultural products are highly susceptible to deterioration (Tahir, 2023). Such losses

are primarily caused by pest infestations, which may result in damage estimated at 26–29% (Novianti et al., 2023). The maize weevil (*Sitophilus zeamais* L.) is one of the most common pests found in maize storage facilities (Arifin et al., 2022; Pramudi & Rosa, 2024). Under optimal environmental conditions, *S. zeamais* L. develops rapidly by feeding on maize kernels and boring into them, thereby causing significant postharvest losses. In addition to quantitative losses, infestation by *S. zeamais* L. reduces nutritional quality, grain weight, and seed germination capacity, particularly in developing countries (Ummah & Ashari, 2025).

Control strategies for storage pests still predominantly rely on chemical methods, particularly synthetic insecticides (Fadillah & Firmansyah, 2025). However, excessive application of synthetic insecticides and fumigants poses serious environmental and food safety concerns (Zhang et al., 2016). Their use can lead to contamination of soil, water, and air, thereby threatening ecological sustainability (Jamin et al., 2024). Consequently, the use of synthetic insecticides should be minimized by adopting safer and environmentally friendly alternatives, such as botanical insecticides (Ngegba et al., 2022).

Botanical insecticides are derived from plant-based active compounds (Saenong, 2016). They are defined as plant-derived chemical substances used to control plant pests, including insects, pathogens, and weeds (Sutriadi, 2019). Botanical insecticides offer several advantages, including the availability of raw materials in the surrounding environment and relatively lower risks to human health (Damalas & Koutroubas, 2020; Ullah et al., 2022). Plants suitable for botanical insecticide development typically contain secondary metabolites such as saponins, tannins, flavonoids, alkaloids, steroids, and essential oils (Tampubolon et al., 2018).

Lime (*Citrus aurantifolia*) leaves are traditionally used as medicinal plants by local communities in Indonesia (Yanuary et al., 2024). Besides their use in traditional medicine, lime fruits are widely utilized in food and beverages. Lime leaves also demonstrate potential as botanical insecticides for pest management (Manullang et al., 2020).

Among communities in Maluku, lime leaves are traditionally utilized due to their bioactive properties and secondary metabolite content. These leaves contain essential oils, flavonoids, saponins, and terpenoids, which exhibit contact and stomach poisoning effects against insects (Saleh et al., 2017; Yudiawati, 2019). Flavonoids also possess antioxidant properties, functioning as free radical scavengers due to the presence of hydroxyl groups. In addition to their medicinal benefits—such as enhancing immunity, improving digestion, alleviating respiratory disorders, reducing cancer risk, preventing diabetes, supporting weight management, maintaining skin health, and preventing anemia (Chhikara et al., 2018; Hartati et al., 2025; Ukratalo, 2025)—lime leaves may also play a role in protecting stored food products from pest infestation, including the maize weevil (*Sitophilus zeamais* L.).

Therefore, this study aimed to evaluate the effectiveness and optimal concentration of lime leaf (*Citrus aurantifolia*) extract on the mortality of the maize weevil (*Sitophilus zeamais* L.).

RESEARCH METHODS

Research Design

This study employed a laboratory-based experimental approach using a Completely Randomized Design (CRD) with five treatments, each replicated three times. The treatments were as follows:

- A0 (Control): 20 g maize + 20 *Sitophilus zeamais* L. adults (without lime leaves)
- A1 (3 g): 20 g maize + 20 *S. zeamais* L. + 3 g lime leaves
- A2 (8 g): 20 g maize + 20 *S. zeamais* L. + 8 g lime leaves
- A3 (13 g): 20 g maize + 20 *S. zeamais* L. + 13 g lime leaves
- A4 (18 g): 20 g maize + 20 *S. zeamais* L. + 18 g lime leaves

Each experimental unit consisted of 20 g of maize grains and 20 adult weevils placed in a plastic container.

Materials and Equipment

The equipment used in this study included an analytical balance, knife, scissors, 15 plastic containers, digital camera, microscope, stopwatch, and stationery.

The materials consisted of young and mature lime leaves (*Citrus aurantifolia*), 300 adult maize weevils (*Sitophilus zeamais* L.), 1 kg of maize grains, label paper, rubber bands, and gauze cloth.

Experimental Procedures

Preparation of Plant Material

Approximately 200 g of lime leaves (*Citrus aurantifolia*) were collected from Galala Village, Sirimau District, Ambon City. The leaves were transported to the laboratory, washed thoroughly, air-dried at room temperature, and cut into small pieces using a knife prior to application.

Preparation of Maize Media

Maize grains (1 kg) were obtained from a storage facility at Mardika Market, Ambon City. The grains were weighed and distributed into treatment containers, with 20 g allocated to each experimental unit.

Preparation of *Sitophilus zeamais* L.

Adult *S. zeamais* L. were collected from infested maize in storage facilities and reared to obtain a uniform-age population. The collected adults were placed in glass jars containing 1 kg of maize grains as a food source. The jars were covered with gauze cloth secured with rubber bands and maintained for four weeks, corresponding to the life cycle of *S. zeamais* L.

After four weeks, all emerged adults were removed. The remaining maize was further incubated for an additional four weeks to obtain newly emerged adults of uniform age. A total of 300 adult weevils were collected and used for the experiment.

Experimental Implementation

Twenty adult *S. Zeamais* L. Were introduced into each treatment container (16 cm height × 14 cm diameter). The experimental steps were as follows:

1. Lime leaves were washed and cut into small pieces.
2. Maize grains were weighed and placed into plastic containers (20 g per container).
3. Lime leaves were added according to the treatment doses (3 g, 8 g, 13 g, and 18 g).
4. Each container was infested with 20 adult maize weevils.
5. The containers were covered with gauze cloth, secured with rubber bands, and labeled accordingly.

Observation Parameters

Observations were conducted over a 24-hour period by recording the number of dead maize weevils in each treatment. Mortality percentage was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead insects}}{\text{Total number of insects}} \times 100$$

Data Analysis

Mortality data were analyzed using Analysis of Variance (ANOVA) with SPSS software. When significant differences were detected, mean comparisons were performed using the Least Significant Difference (LSD) test at a 95% confidence level ($\alpha = 0.05$).

RESULTS AND DISCUSSION

The mortality rates of maize weevils (*Sitophilus zeamais* L.) following treatment with lime leaves are presented in Table 1.

Table 1. Mortality of Maize Weevil (*Sitophilus zeamais* L.)

Observation Time (Hour)	Mortality of Maize Weevil (<i>Sitophilus zeamais</i> L.) (%) at Each Observation Time					Total (Mean ± SD)
	Control	3 g Dose	8 g Dose	13 g Dose	18 g Dose	
0	0.00	0.00	0.00	0.00	0.00	0.00 ± 0.00 ^a
12	0.00	5.00	11.67	20.00	26.67	63.67 ± 10.49 ^b
14	0.00	11.67	16.67	16.67	21.67	66.68 ± 8.38 ^b
16	0.00	15.00	23.33	23.33	20.00	81.66 ± 9.90 ^c
18	0.00	15.00	25.00	31.67	30.00	100.00 ± 12.60 ^d
Total	0,00±0,00^a	46,67±6,51^b	76,00±10,43^c	91,67±11,29^d	98,34±11,41^d	

Note: Means followed by the same superscript letter are not significantly different at $P < 0.05$ (LSD test).

The results presented in Table 1 indicate that the mortality of maize weevils in the control group was 0.00%. In contrast, treatment with lime leaf at a dose of 3 g resulted in a mortality rate of 46.67%, while the 13 g and 18 g doses produced mortality rates of 91.67% and 98.34%, respectively, with the highest dose approaching complete mortality.

In terms of observation time, no mortality was recorded at 0 hours across all treatments, suggesting that the insecticidal effect of lime leaves did not occur immediately after application. However, from 12 to 18 hours of exposure, mortality increased significantly, particularly at higher doses. At 12 hours, the highest mortality rate (26.67%) was observed at the 18 g dose, whereas the lowest dose (3 g) resulted in only 5% mortality.

Mortality continued to increase and reached its peak at 18 hours. At this time point, the 13 g and 18 g treatments resulted in mortality rates of 31.67% and 30.00%, respectively, which were substantially higher than those observed in the control and lower-dose treatments.

Based on the results presented in Table 1, lime leaves (*Citrus aurantifolia*) increased maize weevil (*Sitophilus zeamais* L.) mortality proportionally with the applied dose. Higher concentrations contain greater amounts of bioactive compounds, thereby enhancing toxic potency and accelerating insect mortality. Walkowiak-Nowicka *et al.*, (2023) reported that concentration levels influence the amount of active compounds and consequently affect the onset of insect mortality.

At the initial observation (0 h), no mortality was detected across all treatments, including the untreated control, indicating that the insecticidal compounds did not exert an immediate effect and required time to interact with the insect's physiological system. This pattern is consistent with the characteristics of plant-based insecticides, which generally act more slowly than synthetic pesticides due to their mechanisms of action through contact, inhalation, or ingestion (Anugrah, 2022; Siregar, 2025). After 12 h of exposure, mortality began to increase in a dose-dependent manner, ranging from 5% at 3 g to 26.67% at 18 g. The control group remained at 0% mortality, confirming that insect death resulted solely from the insecticidal treatment. Between 14 and 16 h of observation, mortality increased consistently across treatments, particularly at moderate to high doses (8–13 g), reaching 23.33% at 16 h. These findings demonstrate that the effectiveness of the bioactive compounds increased with both exposure duration and concentration. However, at the highest dose, mortality did not increase linearly, possibly due to saturation of the active compounds within the insect body. This phenomenon is commonly observed in plant-based pesticides, where increasing the dose beyond a threshold does not proportionally enhance toxic effects (Mudjiono, 2013).

After 18 h, cumulative mortality approached 100%, with the 13 g and 18 g treatments resulting in 91.67% and 98.34% mortality, respectively. These results indicate that high doses of lime leaf extract effectively eliminated nearly the entire population of *S. zeamais* within less than 24 h, demonstrating strong potential for postharvest pest management. The sharp increase in mortality suggests a cumulative toxic effect, whereby prolonged exposure progressively disrupts insect metabolic systems.

In general, the mortality of *S. zeamais* in this study can be attributed to limonoid compounds, which exhibit antifeedant properties (Jayatri *et al.*, 2023; Yadnya *et al.*, 2023; Habiba *et al.*, 2024). Antifeedant compounds induce deterrent odors or tastes that inhibit feeding behavior (Pohan, 2024). Reduced food intake significantly affects insects because food serves as the primary source of energy and essential nutrients required for protein synthesis, muscle contraction, and reproduction.

Arim et al. (2018) reported that limonoid antifeedant activity directly suppresses insect feeding, reducing energy availability for metabolic processes. Consequently, essential metabolic pathways are disrupted, including decreased enzyme activity, inhibition of adenosine triphosphate (ATP) production, and failure to maintain physiological homeostasis. These metabolic disturbances impair growth, development, and cellular regeneration, ultimately accelerating mortality due to energy depletion and organ dysfunction.

When limonoids are absorbed and diffuse into the hemolymph—the insect body fluid responsible for nutrient transport, thermoregulation, and immune defense—the toxic effects become systemic (Nisak & Amilah, 2014). Hemolymph dysfunction impairs nutrient and oxygen distribution, weakens immune responses, and disrupts thermoregulation. The combination of these effects triggers systemic metabolic failure, leading to organ dysfunction and eventual death.

In addition to limonoids, other secondary metabolites—including alkaloids, flavonoids, terpenoids, saponins, tannins, and steroids—also contribute to maize weevil mortality. Alkaloids, for example, disrupt the central nervous system (Matsuura & Fett-Neto, 2015). These compounds increase acetylcholine levels by inhibiting acetylcholinesterase activity, resulting in acetylcholine accumulation at synapses. This accumulation induces continuous depolarization through Na⁺ ion influx, leading to excessive positive charge across neuronal membranes. Consequently, nerve impulse transmission to muscle cells is disrupted, causing persistent convulsions, paralysis, and ultimately death (Afifah & Widiyaningrum, 2022).

Flavonoids possess insecticidal properties by inhibiting feeding activity and damaging the nervous system and vital organs due to their toxicity (Harborne & Grayer, 2017; Moniharapon et al., 2020; Zeng et al., 2021; Kaihena & Ukratalo, 2021; Pereira et al., 2024; Ukratalo et al., 2024; Ukratalo et al., 2024a; Sivakumar et al., 2026). Flavonoids also act as respiratory inhibitors. Flavonoids interfere with insect respiration, leading to mortality. Furthermore, flavonoids induce protein denaturation and ATP depletion, thereby disrupting energy and protein metabolism necessary for survival (Riddick, 2024; Syahputri et al., 2024).

Terpenoids exhibit bitter taste and repellent properties and reduce feeding activity (Omar et al., 2007; Koul, 2008; Moniharapon et al., 2019). As antifeedants, they impair food recognition in larvae, resulting in nutritional deficiency. Skopec et al., (2019) further noted that terpenoids may suppress or permanently inhibit feeding behavior depending on their chemical composition.

Tannins exert toxic effects by binding to proteins in saliva and digestive enzymes such as trypsin and chymotrypsin, causing protein inactivation (Shimada, 2006; Da Costa et al., 2008; Moniharapon & Ukratalo, 2021). As a result, insects gradually experience starvation leading to death. Moreover, the complex mixture of secondary metabolites in plant extracts may produce synergistic effects, enhancing insecticidal activity and delaying resistance development (Mohammed, 2014).

Several studies have demonstrated the effectiveness of lime leaves in pest control. Habiba et al. (2024) reported that a 7 g dose with a 14-day observation interval was the most effective treatment against *Sitophilus oryzae*, achieving 100% mortality with no rice damage or

weight loss. Similarly, Pohan (2024) found that a 15 g lime leaf extract treatment (N3) resulted in 82.5% mortality at 21 days after application under laboratory conditions.

Two-way Analysis of Variance (ANOVA) results indicated that both lime leaf dosage and observation time significantly affected maize weevil mortality ($P < 0.05$) (Table 2).

Table 2. Two-Way ANOVA Results

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8918.667 ^a	24	371.611	26.544	0.000
Intercept	11781.333	1	11781.333	841.524	0.000
Treatment	3895.333	4	973.833	69.560	0.000
Observation Time	3495.333	4	873.833	62.417	0.000
Treatment * Time	1528.000	16	95.500	6.821	0.000
Interaction					
Error	700.000	50	14.000		
Total	21400.000	75			
Corrected Total	9618.667	74			

a. R Squared = .927 (Adjusted R Squared = .892)

The LSD test results presented in Table 2 demonstrated that the application of lime leaf extract exerted a significant effect on the mortality of corn aphid pests compared with the control group. The mortality rate observed at the 3 g dose differed significantly from those at the 8 g, 13 g, and 18 g doses, indicating that increasing the concentration of active compounds enhanced the effectiveness of pest control.

The absence of a significant difference between the 13 g and 18 g doses suggests that the effectiveness of the lime leaf botanical insecticide had begun to reach an optimum point. This condition indicates that increasing the dose beyond 13 g no longer produced a significant increase in mortality because the physiological capacity of the insect body to tolerate exposure to toxic compounds had reached its maximum limit. This phenomenon is commonly observed in the application of botanical insecticides, where, after a certain concentration is achieved, additional doses no longer proportionally increase insecticidal activity. According to Zuhro (2024), plant secondary metabolites such as limonoids, flavonoids, and terpenoids possess mechanisms of action that can induce nerve paralysis and metabolic disruption in insects; however, the biological response of insects to increasing concentrations frequently exhibits a plateau or saturation pattern. Furthermore, the high mortality rates recorded at the 13 g and 18 g doses demonstrate that lime leaves have considerable potential as an environmentally friendly alternative bioinsecticide capable of reducing dependence on synthetic insecticides. The use of bioinsecticides is considered safer for the environment because they are more readily biodegradable and pose a lower risk of residue accumulation than synthetic chemical pesticides.

Based on the observation period, corn aphid mortality increased significantly with prolonged contact time between the pests and the lime leaf botanical insecticide. Mortality at 0 h differed significantly from observations at 12 h, 14 h, 16 h, and 18 h, indicating that the active compounds required time to exert their optimal effects within the insect body. The

absence of a significant difference between the 12 h and 14 h observations suggest that, during this interval, the penetration and accumulation of toxic compounds were still occurring gradually. However, at 16 h and 18 h, mortality increased significantly, leading to the conclusion that the toxic effect of lime leaves became more effective after longer exposure periods. This finding is consistent with the study conducted by Ullah et al. (2022), which reported that the effectiveness of botanical insecticides is influenced by both the concentration of active compounds and the duration of contact with target organisms. Prolonged exposure increases the likelihood of active compounds entering the insect body through the respiratory system as well as the integument.

CONCLUSION

Lime leaves (*Citrus aurantifolia*) are effective as a botanical insecticide against maize weevils (*Sitophilus zeamais*), with mortality increasing in response to higher doses and longer exposure times. The most effective dose for controlling maize weevils was 18 g, achieving 98.34% mortality.

The implications of this study indicate that lime leaves can serve as an environmentally friendly alternative for pest control compared with synthetic insecticides. The use of botanical insecticides has the potential to reduce chemical residues in food products, minimize environmental pollution, and decrease the risk of pest resistance resulting from the continuous use of chemical pesticides. In addition, the application of lime leaves as a bioinsecticide may provide an economical solution for farmers because the materials are readily available and relatively safe for non-target organisms.

DECLARATIONS

Author Contributions

C.T contributed to designing the study, preparing samples and test materials, conducting the study, and preparing the manuscript. D. D.M, E.T and M.M contributed to proofreading the manuscript.

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Declaration of Interest

The authors declare no relevant conflicts of interest related to this study.

Data Sharing Statement

The data used in this are available upon reasonable request and in accordance with established procedures.

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