



Original Article

Effect of lime (*Citrus × aurantiifolia* (Christm.) Swingle) and kaffir lime leaves (*Citrus hystrix* DC.) powders on the red flour beetle, *Tribolium castaneum* (Herbst)

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ARTICLE INFORMATION



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ABSTRACT

One of the secondary pests of stored rice is *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Damage caused by this insect includes fungal contamination and the release of benzoquinone-containing secretions, which may pose health risks to humans. Postharvest pest control commonly relies on synthetic fumigant insecticides, yet their use is associated with environmental and health concerns. Plant-derived fumigants offer a more eco-friendly alternative. This study aimed to evaluate the fumigant effects of plant powders from lime (*Citrus × aurantiifolia* (Christm.) Swingle) and kaffir lime leaves (*Citrus hystrix* DC.) on the mortality of *T. castaneum* adult. The experiment employed a untreated, lime leaves, kaffir lime leaves, and a combination of lime + kaffir lime leaves with five replications. Adult mortality was recorded from 3 to 30 days after treatment. Results indicated that by the final observation on day 30, treatment effects were most distinct. Lime leaves (25.67%) and kaffir lime leaves (27.44%) produced significantly higher mortality than both the untreated and the combined treatment ($F_{3,19} = 0.83, P < 0.05$). The combined treatment resulted in only 17.67% mortality, indicating no synergistic effect between the two leaves powders. Mortality in the untreated group remained below 14% throughout the experiment. Lime and kaffir lime leaves, when used individually, exhibit promising fumigant activity against *T. castaneum* and may serve as potential botanical alternatives for postharvest pest management.

Keywords: Adult mortality; Pest control; Plant-derived fumigants; Plant powders; Rice; Secondary pest

1. Introduction

Rice is a staple food for more than 1.6 billion people worldwide, especially in Asia. One of the main challenges in rice postharvest development is pest infestation during storage (Hendriwal et al., 2022). Postharvest pests can cause damage of up to 30–40% (Sharma et al., 2024; Srinivasan et al., 2023). Such infestations lead to a decline in both quality and quantity. One of the secondary pests of rice in storage is *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Rizwan et al., 2024). This insect is cosmopolitan has an extensive host range, a short life cycle, and high tolerance to varying environmental temperatures (Campbell et al., 2022; Majd-Marani et al., 2023). The damage caused by this insect includes fungal contamination and the release of secretions containing benzoquinone compounds, which can pose health risks to humans (Deb & Kumar, 2021).

Postharvest pest control commonly employs synthetic insecticides through fumigation methods (Kim et al., 2019). However, the use of these synthetic insecticides has negative impacts, including pest resistance development and harmful environmental residues (Fan et al., 2025). Currently, environmentally friendly control alternatives are needed. One such eco-friendly approach involves the use of plant-derived powders as botanical insecticides (Mario et al., 2023).

Plant-derived powders from aromatic plants have also gained increasing attention as alternative agents for stored-product pest control due to their content of volatile secondary metabolites (Bedini et al., 2024; Mario et al., 2021). In stored-product insects, these volatile compounds can impair respiratory function, disrupt nervous system signaling, and interfere with digestive processes, ultimately reducing feeding activity and increasing mortality (Piangsuwan et al., 2025). Compared to synthetic

fumigants, botanical-based fumigation is generally more environmentally friendly and poses fewer risks to human health (Kannan & Chandrasekaran, 2026). More than 6.000 plant species belonging to at least 235 families have been reported to possess pest-repellent properties and are traditionally used as botanical pesticides by farmers in various developing countries worldwide (Naimi et al., 2025).

Lime (*Citrus × aurantiifolia* (Christm.) Swingle) and kaffir lime (*Citrus hystrix* DC.) leaves possess strong aromatic properties due to bioactive compounds present in their dried leaves powders, indicating their potential as botanical powders for pest control (Nta et al., 2017). Several studies have reported that both species exhibit strong insecticidal, repellent, and oviposition deterrent activities against a range of stored-product pests. Altazani et al. (2024) demonstrated that kaffir lime extract is effective in controlling *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in stored rice. Similarly, *C. maxima* has been utilized in the management of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) (Rajath et al., 2025), while *C. aurantiifolia* and *C. reticulata* have shown significant bioactivity against *S. zeamais* (Fouad & da Camara, 2017). This study aims to examine the effects of plant powders from lime and kaffir lime leaves when used as fumigants on *T. castaneum* adult.

2. Materials and Methods

2.1. Insect Rearing

The initial insect population of *T. castaneum* were obtained and has been sustained on polished rice (Ciliwung cultivar) in the Entomology Laboratory, Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Hasanuddin since 2019. Rearing was conducted in cylindrical glass containers ($\varnothing = 13$ cm, height = 20 cm) were covered with fine mesh fabric and fastened using elastic bands.

2.2. Grain Materials

The experiment employed polished rice (Ciliwung cultivar) as the test substrate. Sterilization was achieved through sequential cold treatments, consisting of storage at -15 °C for seven days, followed by holding at 5 °C for additional seven-day period. The material was then acclimated under standard laboratory conditions for seven days prior to use (Heinrichs et al., 1985).

2.3. Plant Powders Preparation

The plant materials used lime leaves (*Citrus × aurantiifolia* (Christm.) Swingle) and kaffir lime leaves (*Citrus hystrix* DC.). The leaves samples were air-dried for seven days and ground to a fine powder using an electric blender. For bioassay testing, the plant powder were placed in tea bags at a dose 2 g (Mario et al., 2023).

2.4. Insect Bioassay

This study was conducted using five pairs of *T. castaneum* placed in a plastic container ($\varnothing = 9.9$ cm, height = 6.2 cm) with 90 g of whole rice and 10 g of broken rice under controlled laboratory conditions: 28 ± 2 °C, 33 ± 5 % relative humidity. Plant powder fumigants were added

to the plastic container, which was then sealed to prevent volatile compound loss. The experiment included four treatments: untreated, lime leaves, kaffir lime leaves, and a combination of lime + kaffir lime leaves, each applied at 2 g per container. In the combined treatment, lime leaves and kaffir lime leaves were applied at 1 g each, resulting in a total application rate of 2 g per container. Each treatment was replicated five times, insect mortality was recorded from 3 to 30 days after treatment, and adult mortality using the following formula (1) (Acheampong et al., 2019):

$$\text{Adult mortality (\%)} = \frac{\text{Number of dead adults}}{\text{Number of infested adults}} \times 100\% \quad (1)$$

2.5. Statistical Analysis

The data were obtained using a Completely Randomized Design (CRD). Mortality data were corrected using Abbott's formula (Abbott, 1925) to account for natural mortality in the control treatment, using the following formula (2):

$$\text{Corrected mortality (\%)} = \frac{X - Y}{X} \times 100\% \quad (2)$$

Where X represents adult mortality (%) in control and Y represents adult mortality (%) in treatment. The transformed data were then analyzed using analyzed of variance (ANOVA) at a 5% level of significance. When significant differences among treatments were observed, mean separation was carried out using Fisher's Least Significant Difference (LSD) test at the same significance level. Statistical analysis was performed using Microsoft® Excel version 16.84 and graphical outputs were generated using SPSS® Statistics version 31.0.1.0.

3. Results

Adult mortality of *T. castaneum* increased progressively across all treatments throughout the 30 days observation period. From day 3 to 12, mortality remained low across all treatments, and no significant differences were detected among the untreated, lime leaves, kaffir lime leaves, and combined treatments ($F_{3,19} = 0.32$, $P > 0.05$). By day 15, treatment effects began to appear. Mortality in the lime leaves and kaffir lime leaves treatments started to diverge from the untreated, and statistical analysis indicated significant differences among treatments ($F_{3,19} = 0.38$, $P < 0.05$). At this stage, kaffir lime leaves (15.39%) produced slightly higher mortality than lime leaves (13%), while the combined treatment (13.17%) showed an intermediate response between the single-leaves treatments and the untreated (10%).

More pronounced differences emerged from day 18 onward. On day 18, both lime leaves (15.67%) and kaffir lime leaves (17.61%) resulted in mortality levels that were significantly higher than the untreated (10%) ($F_{3,19} = 0.95$, $P < 0.05$). Kaffir lime leaves consistently produced the highest mortality, whereas the combined treatment continued to yield moderate mortality, remaining lower than the two single-leaves treatments.

By the final observation on day 30, treatment effects were most distinct. Lime leaves (25.67%) and kaffir lime leaves (27.44%) produced significantly higher mortality

than both the untreated and the combined treatment ($F_{3,19} = 0.83$, $P < 0.05$). The combined treatment resulted in 17.67% mortality, consistently lower than either single-leaves treatment, indicating no synergistic interaction

between lime and kaffir lime leaves powders. Throughout the experiment, the untreated showed the lowest mortality, remaining below approximately 14%.

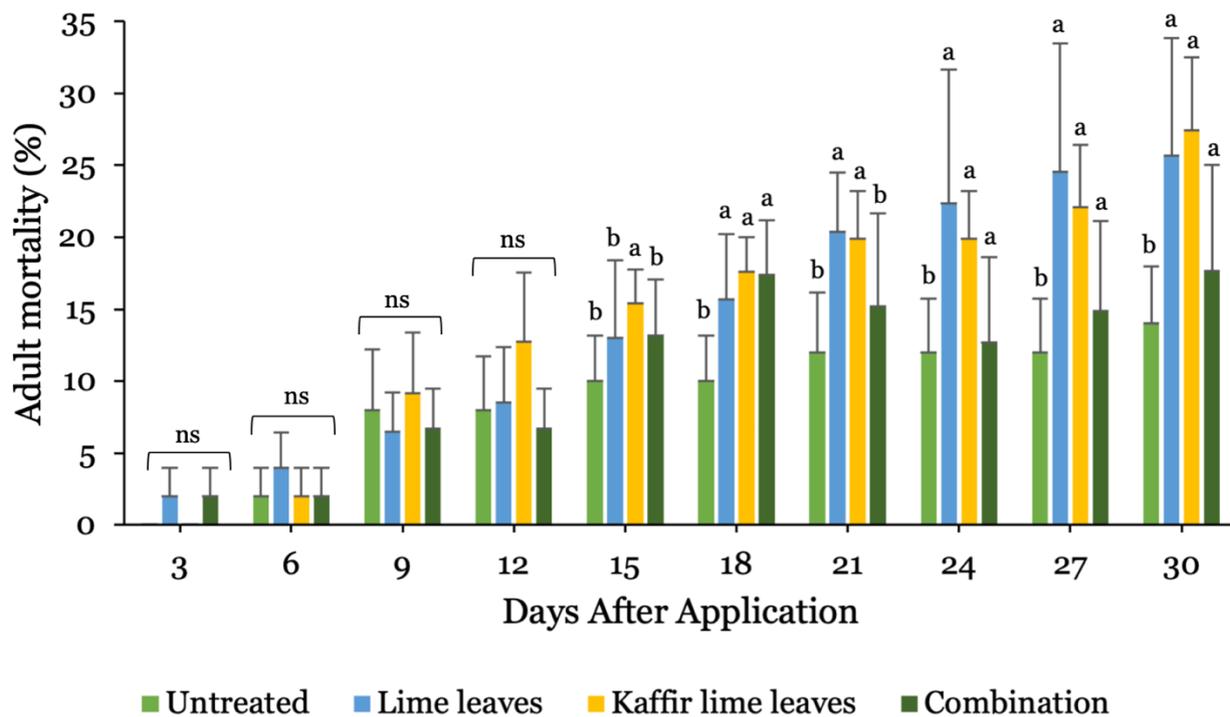


Figure 1. Adult mortality (%) of *Tribolium castaneum* caused by lime and kaffir lime leaves. Data were corrected using Abbott's formula, after which the corrected values were arcsine-transformed prior to statistical analysis. Bar values are expressed as means \pm standard error. Distinct letters above the bars indicate statistically significant differences among treatments within the same day as determined by Fisher's Least Significant Difference (LSD) test ($P < 0.05$), while "ns" denotes non-significant differences ($P > 0.05$).

4. Discussion

The application of lime and kaffir lime leaves powders demonstrated a toxic effect on *T. castaneum*, as indicated by the progressive increase in mortality over the 30-day exposure period. However, the combined treatment of both leaves types produced lower mortality than each leaves applied individually, suggesting that single-leaves applications exhibit higher insecticidal potency. Mortality in the kaffir lime leaves treatment began to emerge on day 15, while lime leaves initiated mortality earlier, indicating a faster toxicity response. By day 30, kaffir lime leaves recorded the highest mortality level (27.44%), followed by lime leaves, whereas the combined treatment remained consistently lower, demonstrating the absence of synergistic interaction between both materials. This finding is consistent with the results reported by Tak & Isman (2017), who stated that combinations of botanical pesticides do not exhibit synergistic effects in controlling *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) in cabbage. This suggests that mixing plant-based insecticidal agents does not necessarily enhance their efficacy and in some cases, may even reduce toxic activity due to interactions or neutralization between active compounds (Babarinde et al., 2018).

The insecticidal activity of citrus leaves is closely associated with their content of volatile secondary

metabolites, which produces a strong aroma that functions as a repellent and disrupts insect behavior (Anggraini et al., 2013). The major compound citronellal plays a central role in inducing toxicity, acting as a contact poison that penetrates the insect cuticle and causes water loss leading to desiccation (Istianah et al., 2013). Lime leaves are also rich in limonoids, which further enhance lethal action and contribute to faster mortality compared to kaffir lime. Conversely, the delayed yet more pronounced mortality effect observed in kaffir lime leaves at later exposure reflects a gradual accumulation of active compounds, indicating that prolonged contact increases toxic efficiency (Hamzah et al., 2024).

Kaffir lime leaves also contain constituents such as D-citronellol, citronellal, -farnesene, and -sinensal compounds known to disrupt cellular metabolism, impair digestive function, and interfere with respiratory activity in stored-product insects this finding is in line with the results reported by Altazani et al. (2024), demonstrated that the bioactive metabolites in kaffir lime leaves are capable of inducing significant mortality in *S. zeamais*. Geraniol, in particular, acts as a stomach poison, preventing food assimilation and leading to starvation-induced mortality (Ogilvie-Battersby et al., 2022). The presence of adult in all treatments beginning on day 21, with the highest abundance in the control and the lowest in the citrus leaves treatments, reinforces the role of these botanical materials

not only as toxicants but also as developmental suppressants (Kim & Lee, 2014).

Previous studies have also demonstrated that citrus-based botanicals effectively suppress several major stored-product pests, including *T. castaneum*, *S. oryzae*, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and *Liposcelis bostrychophila* Badonnel (Psocodea: Liposcelidae), confirming that these volatile compounds play a plant-based insecticidal role in reducing insect survival and postharvest infestation rates (Campolo et al., 2018; Shashikumar et al., 2025; Yadav et al., 2024). The lower effectiveness of the combined treatment in this study may be explained by antagonistic interactions between bioactive compounds, reduced volatilization efficiency when mixed, or decreased concentration of lethal components relative to single-leaves applications (Tak & Isman, 2017).

In addition to its mortality-inducing ability, the use of citrus leaves powders offers several practical advantages for stored-grain protection. These botanical materials are naturally abundant, biodegradable, and environmentally safe, making them suitable alternatives to synthetic insecticides (Ngegbe et al., 2022). Chemical fumigants such as phosphine and pyrethroids, although highly effective, pose risks of resistance development and may leave hazardous residues that threaten food safety and human health (Byrns & Fuller, 2011). In contrast, citrus-based powders release volatile constituents gradually, enabling sustained protection without residue accumulation. This is consistent with previous studies reporting that plant-derived powder exhibits insecticidal and repellent activities, suppresses progeny development, and provides effective grain protection while remaining environmentally friendly (Buatone & Indrapichate, 2011). Thus, the results of this study reinforce the potential integration of citrus leaves formulations into sustainable pest management strategies.

Furthermore, the varying mortality responses observed between single and combined treatments indicate that the efficacy of botanical insecticides may be strongly influenced by compound concentration, interaction, and method of formulation (Khorrami et al., 2018). The reduced effectiveness of the combined lime and kaffir lime treatment suggests that blending plant materials does not always enhance toxicity, and in some cases, may dilute or neutralize active components (Asawalam & Arukwe, 2004). This outcome highlights the importance of understanding phytochemical compatibility when developing multi-plant pest control agents. Future work should therefore explore different mixing ratios, extract-based applications, and encapsulation technologies to enhance volatilization and stability of active constituents. Evaluating sub-lethal parameters such as feeding deterrence, reproduction suppression, larval development, and enzyme disruption may also provide deeper insights into the mode of action of citrus-derived botanicals.

5. Conclusion

The present study confirms that powdered lime and kaffir lime leaves exhibit notable fumigant activity against adult *T. castaneum*, with mortality increasing gradually in

accordance with exposure duration. Among the tested treatments, kaffir lime leaves yielded the highest mortality response, followed by lime leaves, both of which demonstrated statistically significant effects relative to the untreated. Conversely, the combined application of lime and kaffir lime leaves resulted in comparatively lower mortality, suggesting no synergistic enhancement between the two botanicals. These findings indicate that kaffir lime leaves powder represents the most effective botanical material evaluated in this study, while prolonged exposure is essential to maximize insecticidal performance.

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Conflicts of Interest: The authors declare no conflict of interest.

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