

Biological Control Study on Rice Ear Bugs (*Leptocoris oratorius* F.) at Ketindan Agricultural Training Center, East Java, Indonesia

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Abstract

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This laboratory study evaluated the efficacy of two botanical pesticides—lime leaf extract (*Citrus aurantifolia*) and coconut shell liquid smoke—against the rice bug (*Leptocoris oratorius* F.), a major pest in rice cultivation. The research employed a Completely Randomized Design (CRD) with a 4x2 factorial arrangement, testing four concentrations (0% as control, 10%, 20%, and 30%) of each pesticide type. Each treatment combination was replicated three times, with mortality and behavioral observations recorded every 24 hours over 168 hours. Results demonstrated a concentration-dependent effect, with the highest mortality achieved at the 30% concentration. Coconut shell liquid smoke caused 86.67% mortality, outperforming lime leaf extract at 80%. Both treatments induced significant behavioral avoidance, immobilization, and morphological changes such as body darkening and cuticle damage in the pests. The study concludes that both botanical extracts, particularly 30% coconut shell liquid smoke, are highly promising, environmentally friendly alternatives for integrated management of *L. oratorius*, supporting sustainable agricultural practices.

Keywords: botanical pesticide, lime, liquid smoke, mortality, organic pesticide

Introduction

Controlling plant pests using botanical pesticides is an effective approach to controlling or eradicating pests and diseases in cultivated plants (Agadhia et al., 2022; Naftaly et al., 2024). Rice cultivation often faces various problems, with crop failure being one of the main issues caused by pest attacks, resulting in a significant decline in rice production (Kurniawan et al., 2025). One of the dangerous Plant-Incorporated Protectants (PIPs) that can damage grain quality is the rice ear bugs *Leptocoris oratorius* (Hemiptera: Alydidae), which causes discoloration of rice grains and a sharp decline in rice quality (Lahari et al., 2024).

Rice bugs are potential pests on rice plants that become major pests during certain periods. Attacks occur mainly during the milk ripening stage of rice grains, which can reduce grain quality and cause yield losses of up to 50% (Dutta et al., 2016). Plant-based pesticides are one means of controlling pests because they can prevent insects from eating plants, inhibit insect reproduction, disrupt the hormonal system in insects, and control the growth of fungi and bacteria (Arfianto, 2018; Josua et al., 2025). These natural pesticides can be relied upon to overcome pests that have become resistant to synthetic pesticides (Ayilara et al., 2023).

However, plant-based pesticides have several disadvantages, such as rapid decomposition, relatively slow action requiring repeated application, inability to be produced on a large scale, and inability to withstand long-term storage. Plants with potential as plant-based pesticide ingredients must have a strong aroma, bitter taste, be unpalatable to insects/pests, and be usable as medicine (Alprilia et al., 2024). Some plants that can be used include papaya leaves, wedusan/bandotan, garlic, lime leaves, mimba leaves, kipait, mengkudu, soursop leaves, basil, lemongrass leaves, turmeric, and others (Benarivo et al., 2024).

Previous studies have shown the potential of botanical pesticides in controlling rice bugs. Mariana et al. (2024) found that lime leaf solution effectively suppressed rice bug mortality, with an optimal dose of 30

g/70 ml. Meanwhile, Cantika et al. (2023) reported that liquid smoke from coconut shells caused the color of rice bugs to darken or turn blackish brown, with a concentration of 8% being the most effective, where mortality increased with concentration. This research was conducted to investigate the insecticidal potential of locally sourced lime leaf extract and coconut shell liquid smoke, specifically to determine their mortality effects and sublethal impacts on the behavior and morphology of *Leptocorisa oratorius* F. under laboratory conditions.

Materials and Methods

This study was conducted from January 6, 2025, to February 7, 2025, at the Plant Protection Laboratory, Ketindan Agricultural Training Center (BBPP), Jl. Ketindan No.1, Lawang District, Malang, East Java. *L. oratorius* samples were taken directly from farmers' fields by selecting samples for the imago phase. The materials used were 500 grams of lime leaves, 40-50 kg of coconut shells, tissue, labels, water, and rice ear bugs. The tools used in this study were writing instruments, jars, basins, knives, scissors, cutting boards, stirrers, blenders, sieves, hand sprayers, measuring cups, scales, buckets, filter cloths, tissue, plastic bottles, cell phone cameras, funnels, insect nets, sticks, and pyrolysis tools.

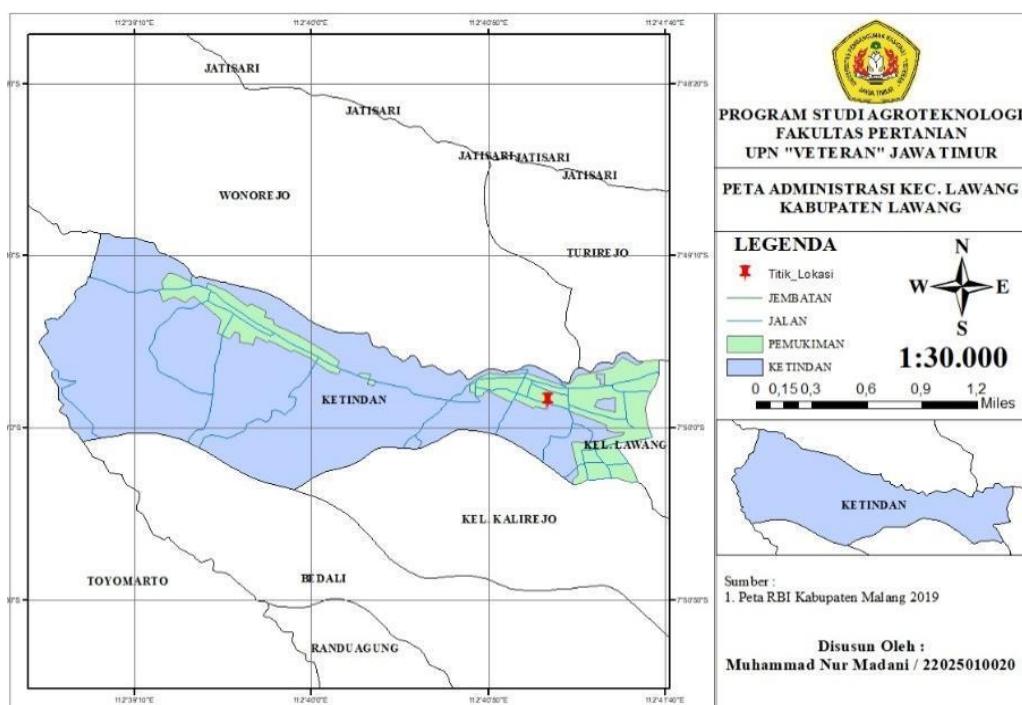


Figure 1. Research location (maps) generated using ArcGis 10.8.

The stages of activities carried out in this study are as follows:

1. Preparation of Natural Pesticide from Lime Leaves

The exploration activity was carried out on land owned by the Ketindan Agricultural Training Center (BBPP). Sample collection focused on the leaves of lime trees. The collection procedure was carried out by picking the leaves directly or using sterile scissors to avoid tissue damage. Leaf collection was recommended in the morning to maintain freshness and active compound content. The selected leaves must be healthy, free from disease spots, and dark green in color, which indicates optimal maturity. Young leaves are avoided because their bioactive compound content is not yet optimal, while old leaves with a dark green color are prioritized to ensure the effectiveness of the extract in controlling pests..

2. Preparation of Tools and Materials for Liquid Smoke Plant-Based Pesticides

The liquid smoke used in this study was produced using a locally assembled pyrolysis apparatus, constructed primarily from repurposed metal drums. The system consists of two main chambers connected by an iron pipe conduit. Coconut shells are selected based on the following criteria: clean of fibers and dirt, cut uniformly (small size), dry (moisture content of 6–9% through natural drying or in an oven at 105°C for 1 hour), and from ripe fruit.

The pyrolysis device design consists of two used drums connected by an iron pipe :

- First drum: A modified drum with perforations at the bottom to allow controlled airflow. A vertical iron pipe is fitted inside to distribute heat evenly from the bottom to the top during combustion. This chamber is loaded with the prepared coconut shells.
- Second drum: A sealed drum functioning as a condenser. It is filled with cold water, which acts as the cooling medium. It is equipped with An inlet pipe that channels hot smoke from the combustion chamber. A collection tube inside to gather the condensed liquid smoke. An outlet pipe to drain the final liquid smoke product.

Operational process consisted of Loading, where dry coconut shells are placed into the Combustion Chamber. Then Sealing, the chamber is tightly sealed, and all gaps are closed to prevent smoke leakage. Pyrolysis where the shells are ignited and smoldered under limited oxygen for 5–7 days, producing dense smoke. After that condensation, The hot smoke travels through the connecting pipe into the Condensation Chamber. Upon contact with the cold water, the smoke condenses into liquid droplets. Final steps, collection. The condensed liquid smoke accumulates and is collected via the outlet pipe.

3. Production of Lime Leaf Extract Pesticide

The production of plant-based pesticide extracts is carried out at the BBPP Ketindan Plant Protection Laboratory. The equipment (bucket, knife, blender) is washed thoroughly and sterilized with 70% alcohol. The lime leaves (500 g) are cleaned, thinly sliced, and then washed again. The leaves are blended with gradual addition of water to a total volume of 2000 ml until smooth. The mixture is left in a closed bucket for 24 hours for extraction of bioactive compounds. The extract is then filtered to separate the pulp. The plant-based pesticide liquid is stored in plastic bottles in a cool, shaded place to maintain stability and shelf life.

4. Production of Liquid Smoke Plant-Based Pesticide

The liquid smoke pyrolysis process begins with sorting used coconut shells to separate the fibers to ensure complete combustion. The shells are then dried in the sun until the water content is low, then broken into small pieces. A total of 40–50 kg of shells are placed in the first drum of the pyrolysis device, tightly closed, and all gaps are sealed to prevent smoke from escaping. Combustion lasts for 5–7 days until the smoke stops. Cold water in the second drum is replaced every 2–4 hours on the first 1–3 days, then adjusted while the embers are still burning, to maintain optimal condensation.

5. Exploration of Rice Ear Bugs

Exploration and sampling of *L. oratorius* F. was carried out in rice fields owned by residents on Jl. Walisongo, Ketindan Village, Lawang District, Malang Regency, East Java, using a manual technique with a sweeping net that was moved across the rice panicles; the trapped samples were carefully transferred to plastic bottles with small holes in the lids to maintain air circulation and survival during transportation, with the number adjusted to the research needs; The samples were then taken to the Ketindan BBPP Plant Protection Laboratory for rearing in special containers with regular rice grain feed, in order to provide sufficient pests and optimal conditions for further testing, such as the effectiveness of plant-based pesticides.

6. Preparation of Natural Pesticide Test Solution Concentrations

Lime leaf extract and liquid smoke are carefully diluted using a measuring cup to achieve concentrations of 0% (control, pure distilled water), 10% (10 ml extract + 90 ml distilled water), 20% (20 ml extract + 80 ml distilled water), and 30% (30 ml extract + 70 ml distilled water) to avoid contamination; the measured solutions were transferred to 100 ml spray bottles, clearly labeled with the type of pesticide, concentration, and date of manufacture, then applied in field trials at 24-hour intervals for 7 days.

Application of Plant-Based Pesticides

The study employed a Completely Randomized Design (CRD) with a factorial arrangement to evaluate the effects of two botanical pesticide types and three concentration levels on the mortality of *L. oratorius*. Factor A: Pesticide Type (J) J1: Lime leaf extract (*Citrus aurantifolia*) J2: Coconut shell liquid smoke. Factor B: Concentration Level (K) K0: Control (0% concentration, distilled water only) K1: 10% concentration K2: 20% concentration K3: 30% concentration. This resulted in 8 treatment combinations J1K0, J1K1, J1K2, J1K3, J2K0, J2K1, J2K2, J2K3. Each treatment combination was replicated 3 times. The experimental unit was a plastic jar containing 5 adult rice ear bugs collected from the field. Therefore, the total number of experimental units was 8 treatments \times 3 replications = 24 jars, with a total of 120 test insects. All 24 experimental jars were labeled and randomly assigned positions within the laboratory observation area to minimize the effect of environmental bias. Each jar received 1 ml of the respective treatment solution, applied evenly using a measuring pipette. Mortality counts and behavioral observations were recorded every 24 hours for a total duration of 168 hours (7 days).

Data Analysis

Observations were made daily for 7 days. Observations were made at 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, and 168 hours after spraying. The parameters observed included changes in the behavior of *L. oratorius* after the application of botanical pesticides and the number of dead *L. oratorius*. The observations were conducted by calculating the mortality rate of *L. oratorius* (%). The calculation was performed by counting the number of dead *L. oratorius* each day, starting from 24 hours after pesticide application up to 168 hours. According to Piepho et al. (2024), the percentage of pest mortality can be calculated using the following formula:

Description:

TM = Percentage of *L. oratorius* mortality

UA = Number of dead *L. oratorius*

UT = Total number of *L. oratorius*

The primary data collected was the cumulative number of dead insects per jar at each observation interval. Mortality percentage was calculated using Abbott's formula if control mortality exceeded 5%. Data were analyzed using Analysis of Variance (ANOVA) for a factorial CRD. If the ANOVA indicated significant effects ($p < 0.05$), the treatment means were further compared using Duncan's Multiple Range Test (DMRT) or an appropriate post-hoc test to identify which specific treatments differed significantly.

Result and Discussion

Application of Botanical Pesticides

Management of *L. oratorius* F. pests at the Ketindan Agricultural Training Center utilizes plant-based pesticides derived from lime leaf extract and coconut shell liquid smoke as an environmentally friendly alternative to synthetic pesticides. Plant-based pesticides, defined as natural chemical compounds from plants, offer advantages such as the availability of local raw materials, ease of manufacture, low cost, and high biodegradability, so that residues disappear quickly and are safe for consumers (Wibowo et al., 2022).

Their mechanism of action includes a repellent effect through a pungent aroma, an antifeedant effect due to a bitter taste, and physiological disruption of insects (Sari et al., 2024). The effectiveness of lime leaf extract is supported by its diverse secondary metabolite content, including alkaloids, flavonoids, limonoids, essential oils, saponins, and tannins (Huda, 2018). Limonoids disrupt the insect's hormonal system and growth; essential oils (citral, limonene, linalool) function as insecticides and repellents by disrupting the nervous system; flavonoids inhibit appetite; saponins damage cell membranes; and tannins bind digestive enzymes (Dumanauw et al., 2019). This combination results in high mortality with a broad spectrum of action.

Meanwhile, coconut shell liquid smoke a pyrolysis product contains phenolic compounds (guaiacol, syringol), acids (acetate, propionate, formate), and carbonyls (furfural) (Cantika et al., 2023). Phenolic compounds damage the cuticle and nervous system; acids disrupt metabolism and digestion; while carbonyls act as repellents by inhibiting olfactory receptors, making it difficult for rice bugs to detect host plants. The synergy of these three groups of compounds enhances their lethality and prevents attacks. The integration of lime leaf extract and coconut shell liquid smoke offers an effective, selective, and sustainable integrated control strategy. This approach not only significantly suppresses rice bug populations but also supports environmentally friendly agriculture by minimizing the risk of resistance and ecosystem contamination.

Mortality Rate of Rice Ear Bugs

Observations over 7 days showed that plant-based pesticides derived from lime leaf extract (J1) and coconut shell liquid smoke (J2) significantly affected the mortality of *Leptocoris oratorius*, with toxic effects increasing with concentration and exposure time (Figure 2.). On the first day (24 hours), no significant mortality was observed in all treatments, indicating a latent period before the active compounds had a lethal effect. Starting on the second day, the highest mortality was recorded in J1K3 (30% lime extract) at 20%, while J2K3 (30% liquid smoke) dominated from the third day until the end with a peak of 86.67% on the seventh day, followed by J1K3 at 80%. These findings indicate that liquid smoke at a concentration of 30% is the most effective treatment, reaching a lethal threshold of >80% according to the criteria for potential insecticides.

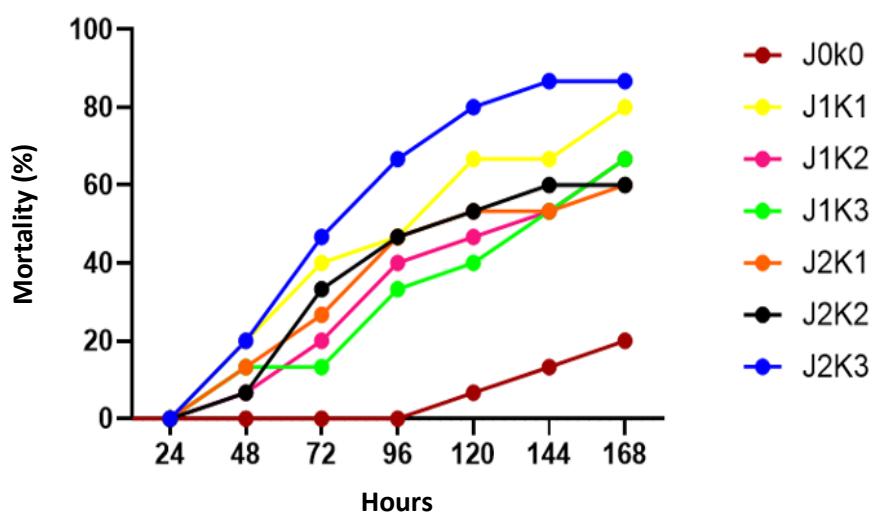


Figure 2. Graph of Mortality Percentage of *L. oratorius*.

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The superiority of J2K3 is supported by its dominant phenolic compound content (86.67%), such as guaiacol and syringol, which damage the cuticle, disrupt the nervous system, and inhibit cell respiration (Hadano & Apituley, 2016; Anom & Mamangkey, 2016). Acidic compounds (acetate, formate) cause metabolic acidosis, while carbonyl compounds (furfural) act as *repellents* and enzyme inhibitors (Isa et al., 2019). The absorption mechanism through the intestinal wall allows the active compounds to reach physiological targets systemically (Vikram, 2018), resulting in mortality reaching >50% within 5 days and stabilizing at 86.67% by the seventh day.

Treatment J1K3 showed a similar pattern but slightly slower, with a final mortality rate of 80%. This effectiveness is associated with the synergy of secondary metabolites: limonoids disrupt moulting and reproduction, essential oils (limonene, citral) act as neurotoxins and *repellents*, flavonoids and tannins act as *antifeedants*, and saponins lyse cell membranes (Huda, 2018; Mawuntu, 2016). The increase in mortality with dose confirms a dose-response relationship consistent with the principles of botanical insecticide toxicology. Both treatments at a concentration of 30% were significantly superior to the control and low concentrations, confirming that the effective toxicity threshold was reached at this level. The stability of J2K3 mortality after the sixth day indicated a rapid but short-lived residual effect, consistent with the biodegradable nature of botanical pesticides. These findings reinforce the potential of coconut shell liquid smoke and lime leaf extract as effective, selective, and environmentally friendly alternatives for controlling rice bugs in sustainable agricultural systems.

Behavior of Rice Ear Bugs Before and After Application

The application of plant-based pesticides derived from coconut shell liquid smoke and lime leaf extract triggered significant changes in the behavior, physiology, and morphology of *Leptocoris oratorius*, reflecting diverse bioactive action mechanisms. Before treatment, leafhoppers exhibited high activity with rapid movement and a preference for resting on container lids, indicating healthy conditions and normal exploration. After spraying, an immediate defensive response was observed: in the case of liquid smoke, individuals became immobile with hyperactive antenna movements, presumably as an attempt to detect threats and avoid contaminated areas due to nervous system disruption by phenolic compounds (Isa et al., 2019). Phenol damages the cuticle, increasing susceptibility to dehydration and infection, while acetic acid induces metabolic disorders and damage to the digestive tract (Vikram, 2018). Carbonyl compounds act as *repellents* by disrupting olfactory receptors, inhibiting host seeking (Juwita, 2019). This combination not only suppresses mobility but also accelerates physiological collapse.

In lime leaf extract, rice bugs exhibit evasive behavior by avoiding the spray point, driven by the strong aroma of essential oils (limonene, citral) that act as *repellents* (Kasi, 2012). Alkaloid compounds disrupt moulting and metamorphosis (Huda, 2018); flavonoids inhibit brain hormones, ecdysone, and growth (Dumanauw et al., 2019); limonoids act as stomach poisons causing weakness; saponins lyse cell membranes; and tannins bind digestive proteins (Gogok, 2013). This synergy produces *antifeedants* and continuous developmental disruption.

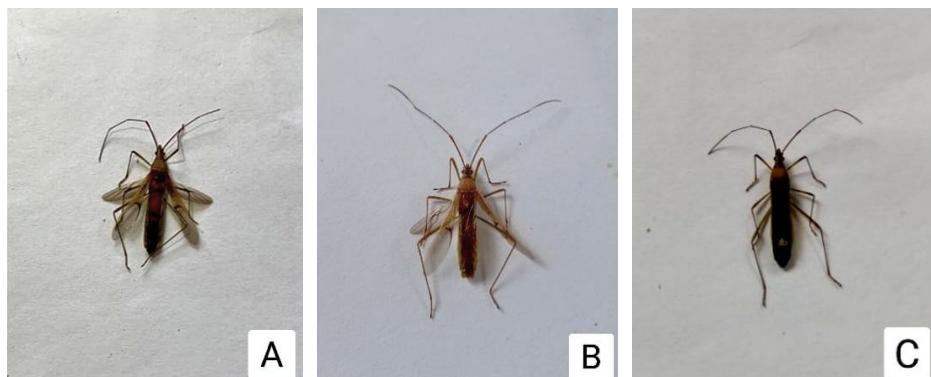


Figure 3. Comparison of Insect Skin Color After Application, (A) Lime Leaf Extract; (B) Control; (C) Liquid Smoke Extract

Post-treatment morphological changes reinforce the differences in action mechanisms. Control individuals were greenish-yellow (Figure 3B); after lime extract application, the body and wings turned reddish-brown to dark brown, indicating phenolic oxidation and protein denaturation (3A). In liquid smoke (3C), the changes were more extreme: blackish brown color accompanied by stiffness and structural fractures, reflecting severe cuticle damage by phenols and tissue acidification (Isa et al., 2019). This damage confirms that liquid smoke has a more destructive spectrum of action than lime extract, which tends to be systemic. Figure 4 provides visual evidence of the profound sublethal effects induced by the tested botanical pesticides on *L. oratorius*, directly supporting the physiological and behavioral disruptions discussed in the results. This image illustrates a failed molting process, where the insect is unable to fully shed its old exoskeleton. This observation is a direct consequence of the hormonal and developmental interference caused by the bioactive compounds in the pesticides (Campli et al., 2024). As discussed, limonoids in lime leaf extract are known to disrupt the insect's hormonal system, particularly affecting ecdysone, the hormone responsible for molting and metamorphosis. Similarly, the metabolic acidosis induced by acidic compounds (e.g., acetate, formate) in liquid smoke can impair the enzymatic and energetic processes necessary for successful ecdysis. This disrupted molting leaves the insect vulnerable, weakened, and often leads to death, confirming the insect growth regulator (IGR)-like activity of these botanical extracts (Fernando et al., 2024).

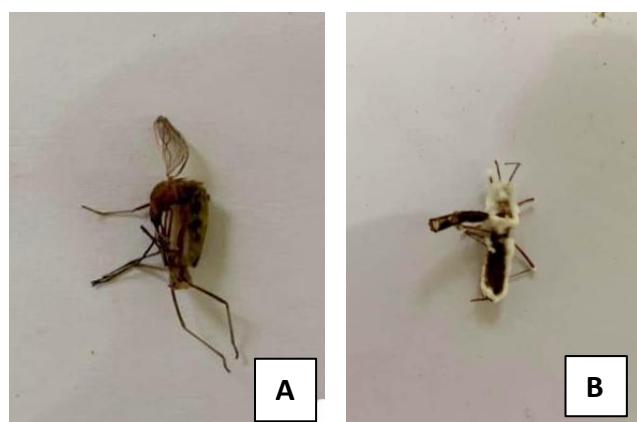


Figure 4. Morphological Changes in Insects After Application of Plant-Based Pesticides, (A) Disruption of Ecdysis; (B) Decay and Loss of Body Parts.

Conclusion

Lime leaf extract and coconut shell liquid smoke have been proven to have potential as effective botanical pesticides for controlling *Leptocoris oratorius* F. The J2K3 (30% liquid smoke) and J1K3 (30% lime) treatments achieved the highest mortality rates of 86.67% and 80%, respectively, 168 hours after application. Both extracts also induced significant behavioral changes, including direct avoidance, immobilization, weakness, slow movement, and moulting disorders, reflecting disruption of the pest's nervous system, metabolism, and physiological integrity. changes in evasive behavior and immobilization, combined with morphological degradation, indicate that both botanical pesticides are effective through multiple targets:

disruption of the nervous system, metabolism, digestion, and structural integrity. Liquid smoke excels in acute and destructive effects, while lime extract is more effective in long-term inhibition, supporting a rotation strategy to prevent resistance. These findings reinforce their potential as environmentally friendly alternatives in integrated pest management of rice bugs. Field trials and farmer training programs should be conducted to validate and promote their adoption within integrated pest management systems. Furthermore, policy support and further research into synergistic formulations are recommended to ensure sustainable and widespread implementation.

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