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Bioefficacy of Eugenol-Based Bioinsecticide from Clove Leaf Oil (*Syzygium aromaticum*) against Brown Planthopper (*Nilaparvata lugens*)

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Abstract. The brown planthopper (*Nilaparvata lugens*) is one of the most destructive pests of rice, causing significant yield losses and threatening global food security. The extensive use of synthetic insecticides has led to environmental concerns and the development of insecticide resistance, highlighting the need for sustainable alternatives. This study aimed-to purify eugenol from clove leaf oil (*Syzygium aromaticum*), formulate a stable oil-in-water bioinsecticide emulsion, and evaluate-its insecticidal and antifeedant activities against *N. lugens*. Eugenol was enriched through vacuum fractional distillation and characterized using GC-MS, GC, FTIR, and physicochemical analyses. The purified eugenol was subsequently formulated using Tween 80 and ethanol, and its biological activity was assessed through toxicity and honeydew excretion bioassays. Vacuum fractional distillation increased eugenol content from 53.53% to 94.30%. Clove oil and purified–eugenol exhibited LC_{50} values of 0.016% and 0.019%, respectively. In definitive toxicity assays, clove oil caused 90.00% mortality at 0.120%, whereas purified eugenol produced 73.33% mortality at 0.076% after 96 h of exposure. In addition, purified eugenol reduced feeding activity by up to 60%, demonstrating pronounced antifeedant effects. These findings indicate that eugenol contributes to both lethal and sublethal suppression of *N. lugens* and highlight the potential of clove-derived bioinsecticides as environmentally compatible alternatives–for sustainable rice pest management. The study further provides a value-added strategy for utilizing clove leaf oil and contributes to the development of SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land).

Keywords: Clove Oil; eugenol; botanical insecticide; brown planthopper; antifeedant activity

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INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food for more than half of the global population and plays a fundamental role in food security, particularly across rice-dependent regions of tropical Asia (Dollison et al., 2025). However, rice production remains highly vulnerable to insect pests, among which the brown planthopper (*Nilaparvata lugens* Stål; Hemiptera: Delphacidae) is one of the most destructive (Jr et al., 2023; Prihandiani et al., 2021). This pest causes severe damage through continuous phloem sap feeding, leading to hopperburn, and indirectly by transmitting rice grassy stunt virus and rice ragged stunt virus. During severe outbreaks, infestations may result in yield losses ranging from 20% to complete crop failure, posing a major threat to regional food security (Rafiq et al., 2022).

Synthetic insecticides have long been the primary strategy for managing *N. lugens* in Asian rice production systems. Although highly effective, their intensive and prolonged use has led to widespread insecticide resistance, particularly to neonicotinoids such as imidacloprid, thiamethoxam, and clothianidin, thereby reducing field efficacy and increasing production costs (Datta et al., 2021). Moreover, excessive pesticide use has disrupted natural enemy populations, promoted pest resurgence, contaminated paddy ecosystems, harmed non-target organisms, and increased risks to human health through occupational and dietary exposure. These challenges highlight the urgent need for sustainable and environmentally compatible pest management strategies within the framework of Integrated Pest Management (IPM).

Botanical insecticides derived from plant secondary metabolites have emerged as promising alternatives because they are biodegradable, environmentally safer, and possess diverse modes of action that reduce the likelihood of resistance development (Inocencio et al., 2025; Lengai et al., 2020). Among these, clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry) essential oil has attracted considerable attention due to its high eugenol content, typically accounting for 70–90% of the oil composition. Eugenol exhibits contact insecticidal, fumigant, repellent, and antifeedant activities against numerous insect pests. Its insecticidal activity has been attributed to inhibition of acetylcholinesterase, disruption of octopaminergic neurotransmission, and interference with insect metabolism, leading to feeding inhibition, paralysis, and mortality. Unlike neonicotinoids, which primarily target nicotinic acetylcholine receptors, eugenol acts through multiple physiological pathways, suggesting a lower risk of resistance development and making it a promising candidate for IPM programs targeting *N. lugens* (Sarma & Khanikor, 2025; Tursiloadi et al., 2017).

Despite their potential, essential oils have limited agricultural applications due to their high volatility, poor water solubility, and susceptibility to oxidation, heat, and photodegradation, all of which reduce their persistence and bioavailability under field conditions (Santiago-Santiago et al., 2024). Two complementary approaches can overcome these limitations. Vacuum fractional distillation enables enrichment of high-purity eugenol from crude clove oil, providing standardized active ingredient content and improved reproducibility. Meanwhile, formulation as an oil-in-water (O/W) emulsion enhances aqueous dispersibility, colloidal stability, and controlled release, thereby improving persistence and insecticidal performance under field conditions (Do & Le, 2026; Zhang et al., 2025).

Recent studies have demonstrated the effectiveness of essential oil nanoemulsions against various agricultural pests. For example, *Eucalyptus globulus* nanoemulsions showed strong toxicity against *Sitophilus oryzae* and *Tribolium castaneum* (Adak et al., 2020), *Salvia officinalis* nanoemulsions were effective against *Sitophilus granarius* (Nenaah et al., 2023), *Piper tuberculatum* nanoemulsions controlled *Bemisia tabaci* (Rodrigues et al., 2025), *Mentha spicata* and carvone nanoemulsions suppressed *Rhopalosiphum maidis* and *Sitobion avenae* (Mondal et al., 2024), while *Callistemon citrinus* nanoemulsions produced high mortality against the rice stem borer *Chilo suppressalis* (Haghbayan et al., 2024). Nevertheless, most previous studies have relied on crude essential oils without standardization of active compounds and have evaluated only a single biological endpoint. To date, no study has investigated both insecticidal and antifeedant activities of an eugenol-enriched emulsion against *N. lugens*.

Therefore, this study aimed to (1) purify eugenol from clove leaf essential oil using vacuum fractional distillation, (2) develop and characterize a stable oil-in-water emulsion, and (3) evaluate its insecticidal and antifeedant activities against *N. lugens*. The findings are expected to provide scientific evidence supporting the development of standardized botanical insecticides as environmentally sustainable alternatives for rice pest management.

METHODS

A quantitative laboratory experimental approach was adopted to evaluate the physicochemical properties and insecticidal efficacy of eugenol extracted and purified from *S. aromaticum*. The experimental units consisted of purified eugenol, oil-in-water emulsion formulations, and *N. lugens* nymphs. Primary data were obtained through standardized laboratory bioassays and physicochemical instrumentation; descriptive statistics and probit analysis were applied to estimate LC_{50} and LC_{95} values. A schematic overview of the research workflow is presented in Fig. 1.

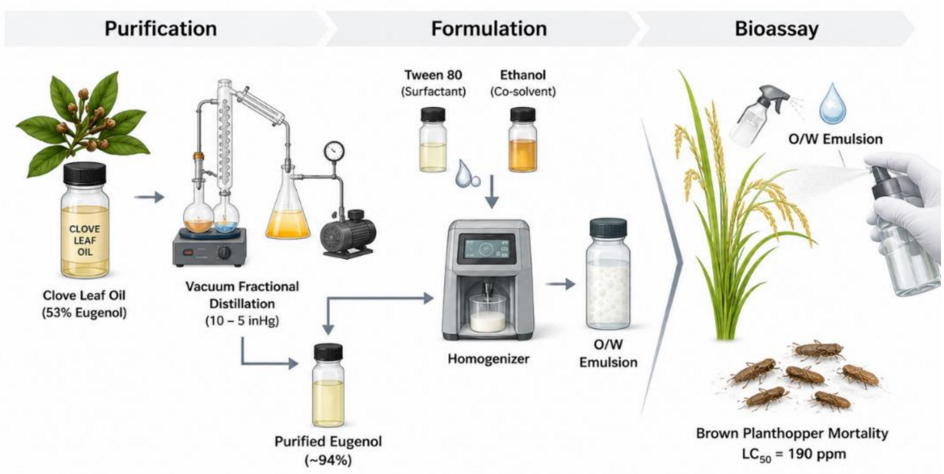

Figure 1. Overview of the three-stage experimental workflow: eugenol purification from clove leaf oil (53% eugenol) by vacuum fractional distillation (10-5 inHg) to ~94% purity; formulation of an oil-in-water (O/W) emulsion using Tween 80 (surfactant) and ethanol (co-solvent) via homogenization; and bioassay-based evaluation of insecticidal activity against *N. lugens*, yielding an LC_{50} of 190 ppm.

Purification and Quality Characterization of Eugenol

Crude eugenol from *S. aromaticum* clove leaf oil was purified by vacuum fractional distillation using a 300 mL charge in a three-neck round-bottom flask fitted with a fractionating column, condenser, thermometer, and vacuum system. Distillation was performed at 120 °C under 10 inHg, followed by redistillation of selected fractions at 5 inHg to maximize purity while minimizing thermal degradation (Do & Le, 2026). Purified eugenol was characterized by GC and GC–MS, while physicochemical properties were evaluated according to SNI 06-2387-2006, including organoleptic characteristics, density, refractive index, and solubility in 70% ethanol (Pujiarti et al., 2020).

Formulation and Characterization of Eugenol-Based Bioinsecticide

Purified eugenol from *S. aromaticum* was formulated into oil-in-water (O/W) emulsions at 0, 50, 100, 150, and 200 ppm using Tween 80 (0.2%, w/v) and ethanol (1%, v/v) as surfactant and solvent. Eugenol was dissolved in a Tween 80 ethanol mixture (5:1, v/v), diluted with distilled water to a final volume of 100 mL, and homogenized to obtain stable emulsions (Kamaraj et al., 2024). Physicochemical characterization included organoleptic properties, pH, emulsion type, and physical stability. FTIR analysis was performed to identify functional groups and evaluate intermolecular interactions between eugenol and formulation components (Kiromah et al., 2023).

Bioinsecticidal Activity Assay

Preparation of Rice Plants and Insect Test Organisms

Rice (*O. sativa*) cv. Ciherang was grown under controlled laboratory conditions as the host plant for bioinsecticidal assays. Seeds were soaked in distilled water for 24–48 h, sown in moist paddy soil, and maintained for 21 days. Healthy seedlings (22–25 cm tall with 5–6 fully expanded leaves) were selected to ensure uniformity. Second-instar nymphs of the brown planthopper (*Nilaparvata lugens*) were obtained from established laboratory colonies at the Laboratory of Pest Control Technology–R. Biological, Faculty of Agriculture, Universitas Gadjah Mada. This stage was selected because of its active feeding behavior and susceptibility to insecticides. Each experimental unit contained ten nymphs with treated rice seedlings in ventilated chambers.

Preliminary Toxicity Test

Preliminary toxicity bioassays were conducted using a contact exposure method to determine the susceptibility of second-instar *N. lugens* nymphs and establish the concentration range for subsequent assays. Eugenol formulations (0.005%, 0.010%, 0.015%, and 0.020%) and a formulation-only control were tested in triplicate. Ten nymphs per replicate were sprayed with 0.4 mL of each treatment, and mortality was recorded at 24, 48, 72, and 96 h. Dose–response data were analyzed using probit regression to estimate LC_{50} and LC_{95} values, along with their corresponding confidence intervals

(Gueretz et al., 2017; Vasconcelos et al., 2024).

Advanced Toxicity Test

Based on the preliminary toxicity assay, four biologically effective eugenol concentrations were selected for advanced toxicity evaluation with five independent replicates. Mortality data were analyzed by probit regression using POLO-PC software (LeOra Software, Berkeley, CA, USA) to estimate LC_{50}, LC_{95}, slope values, and 95% confidence intervals. Model goodness-of-fit was evaluated using the chi-square test, where non-significant values indicated adequate agreement between observed and expected mortality responses.

Antifeedant Activity Test

Antifeedant activity was evaluated using a honeydew excretion bioassay as an indirect measure of feeding by *N. lugens* (He et al., 2013). Rice seedlings were treated with eugenol at LC_{25}, LC_{50}, and LC_{75}, while controls received the formulation matrix only. After spray deposits had dried, three fourth-instar nymphs were introduced into each ventilated chamber. Honeydew was collected on pre-weighed parafilm sheets for 48 h, quantified gravimetrically, and expressed as the percentage reduction in feeding relative to the control (Nasution et al., 2021). Antifeedant activity was expressed as the percentage reduction in feeding relative to the control treatment and was calculated according to Equation(1):

$$PM = \frac{HC-HT}{HC} \times 100\% \qquad (1)$$

where PM represents the percentage of feeding inhibition (%), HC denotes the mean weight gain of parafilm in the control treatment (mg), and HT denotes the mean weight gain of parafilm in the treatment group (mg).

RESULTS AND DISCUSSION

This study presents an integrated approach combining vacuum fractional distillation, emulsion formulation, and biological evaluation to develop a clove (*Syzygium aromaticum*) leaf oil-based bioinsecticide against *N. lugens.* Vacuum fractional distillation increased eugenol content from 53.53% to 94.30%, yielding a purified fraction with enhanced insecticidal performance. Both purified eugenol and crude clove leaf oil exhibited significant insecticidal and antifeedant activities, demonstrating their potential as sustainable alternatives to synthetic insecticides in integrated pest management (Zhang et al., 2026). Comparative evaluation showed that higher eugenol purity improved efficacy, whereas the retained activity of crude oil suggests additive or synergistic effects of minor constituents. These findings support the development of effective and environmentally compatible plant-based insecticides.

Physicochemical Characterization of Clove Leaf Oil and Purified Eugenol

Prior to vacuum fractional distillation, the physicochemical properties of crude clove leaf oil were evaluated to confirm compliance with SNI 06-2387-2006 and its suitability for purification. The oil met all quality specifications, exhibiting a yellowish-brown color, a characteristic clove aroma, a density of 1.0309 g/mL at 20 °C, and complete solubility in 70% ethanol (1:2). These characteristics indicate high-quality clove oil and the preservation of its volatile phenolic constituents (Mahulette et al., 2020). The density and solubility are consistent with those of previously reported eugenol-rich clove oils, confirming the quality and reliability of the raw material (Suttiarporn et al., 2025).

GC–MS, GC, and FTIR analyses were employed to characterize the compositional profile of clove leaf oil and to evaluate the efficiency of eugenol enrichment achieved through multistage vacuum distillation (**Fig. 2**). The crude oil was dominated by eugenol (53.53%), with trans-caryophyllene (40.30%) representing the major accompanying sesquiterpene, together with smaller proportions of α-humulene and caryophyllene oxide (Sohilait et al., 2023). Following vacuum redistillation, the eugenol content increased markedly to 94.30%, while the relative abundance of sesquiterpene constituents decreased substantially. This pronounced shift in chemical composition confirms the effectiveness of vacuum distillation as a selective purification strategy for concentrating eugenol from complex essential oil matrices (Abdou et al., 2025).

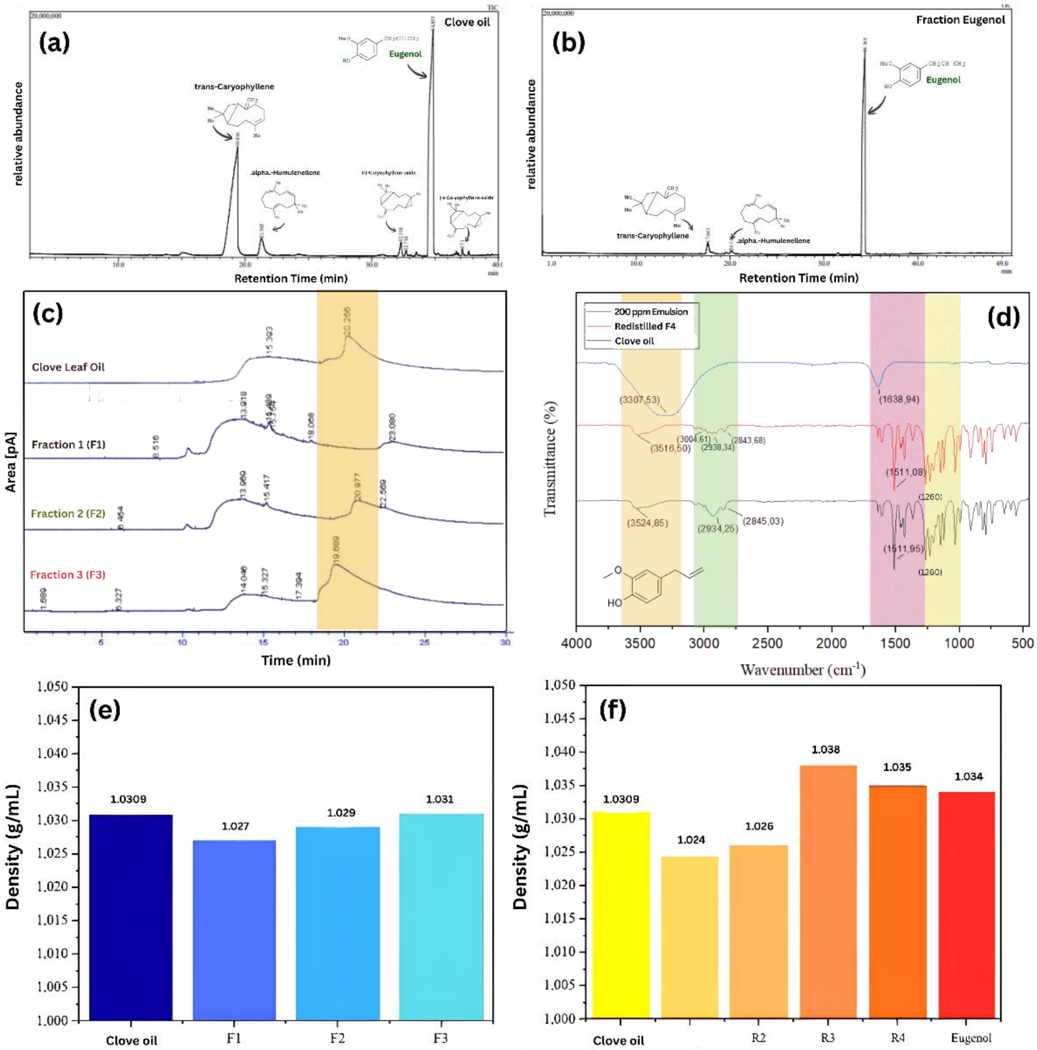

Figure 2. Physicochemical characterization: (a) GC–MS of clove leaf oil, (b) GC of redistilled eugenol, (c) GC of eugenol fractions, (d) FTIR spectra, (e) density of eugenol fractions, and (f) density of redistilled fractions.

The enhanced separation efficiency can be attributed to operation under reduced pressure (10 inHg), which lowered the boiling temperatures of volatile components and increased the volatility differences among constituents. Consequently, eugenol could be recovered with minimal co-distillation of higher-boiling sesquiterpenes while avoiding excessive thermal exposure. Preservation of the phenolic structure of eugenol is particularly important because its hydroxyl and methoxyl functional groups are closely associated with its insecticidal and antifeedant activities. Therefore, the substantial increase in eugenol purity observed after redistillation is expected to contribute directly to the improved biological performance of the resulting bioinsecticide formulation.

The GC chromatographic profiles of the distillation fractions (Fig. 2b) further validated the GC-MS findings, demonstrating a progressive enrichment of eugenol throughout the multistage purification process. Fraction 3 (F3) exhibited the highest peak intensity within the characteristic eugenol retention window, indicating the greatest degree of purification among all fractions. In contrast, peaks corresponding to sesquiterpene constituents decreased substantially following successive distillation steps. The observed fractionation behavior can be attributed to differences in physicochemical properties among the oil constituents. Sesquiterpene hydrocarbons, which generally possess lower polarity and greater volatility, were preferentially removed during the earlier stages of distillation. Conversely, eugenol accumulated in the later fractions due to its higher boiling point and the presence of a phenolic hydroxyl group capable of intermolecular hydrogen-bonding interactions, thereby reducing its volatility (Munteanu et al., 2024).

FTIR analysis (Fig. 2c) provided further confirmation of the compositional changes observed during vacuum distillation. Both crude clove leaf oil and purified fractions exhibited the characteristic spectral features of eugenol, including broad O–H stretching vibrations (3300–3500 cm^{-1}), aliphatic C–H stretching bands (~2930 cm^{-1}), aromatic C=C skeletal vibrations (~1510 cm^{-1}), and aromatic ether C–O stretching bands within the 1200–1300 cm^{-1} region. The purified eugenol fraction showed noticeably sharper and more intense absorption bands than the crude oil, reflecting a higher relative abundance of eugenol and reduced contributions from co-existing volatile constituents (Ahamad et al., 2022). Importantly, no substantial shifts in the positions of the characteristic bands were observed, indicating that vacuum redistillation selectively removed impurities while preserving the fundamental molecular structure of eugenol. This structural preservation is essential for maintaining the biological activity associated with its phenolic and aromatic functional groups.

Density measurements provided complementary physicochemical evidence for the effectiveness of the vacuum redistillation process (Fig. 2d). The density of the purified fraction progressively approached that of pure eugenol (approximately 1.034 g/mL), indicating a substantial increase in the relative abundance of oxygenated phenolic compounds and a reduction in hydrocarbon-rich sesquiterpene constituents. Such changes are consistent with the chromatographic profiles showing eugenol enrichment and the FTIR spectra demonstrating preservation of characteristic phenolic functional groups. Furthermore, the purified fraction exhibited complete solubility in 70% ethanol at a ratio of 1:2. This behavior can be attributed to the semi-polar character of eugenol and the ability of its phenolic hydroxyl group to participate in hydrogen-bonding interactions with ethanol molecules, thereby promoting miscibility in polar organic solvents (Abdou et al., 2025; Mahulette et al., 2020).

Stability and Characteristics of EO-Bioinsecticide

Eugenol emulsions containing ≤200 ppm remained optically clear, homogeneous, and free from phase separation, creaming, or sedimentation during four weeks of storage, with a stable pH of 5.8–6.2 (Fig. 3). This stability demonstrates the effectiveness of the Tween 80–ethanol system in reducing interfacial tension and stabilizing dispersed eugenol droplets. In contrast, formulations containing >330 ppm exhibited turbidity and phase separation, likely due to insufficient surfactant coverage, leading to droplet aggregation and coalescence. Similar concentration-dependent instability has been reported for essential oil emulsions (Suriyarak & Weiss, 2014).

All formulations consistently formed oil-in-water (O/W) emulsions, as confirmed by the dye distribution test (Fig. 3). O/W systems provide efficient aqueous dispersibility, enabling uniform distribution of eugenol droplets during spray application. Enhanced wettability and spreading on leaf surfaces may improve disposition and retention of the active ingredient, thereby increasing its accessibility to feeding and contacting insects. Consequently, the physicochemical properties of the developed emulsion are expected to enhance the biological performance of the bioinsecticide, particularly under practical application conditions.

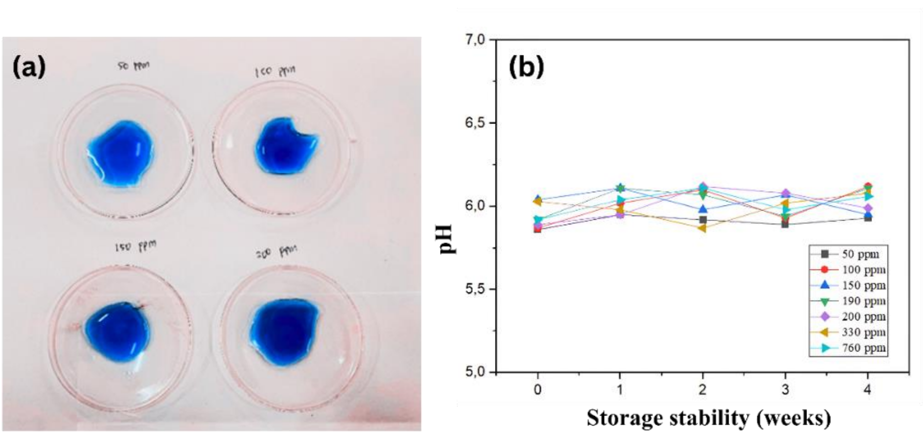

Figure 3. Stability evaluation of the emulsion (a) emulsion type determination test, and (b) pH test for storage stability assessment

Insecticidal and Antifeedant Activity of Clove Leaf Oil

Toxicity Test

Following physicochemical characterization and purification, the biological efficacy of clove leaf oil and the eugenol-enriched Fraction 3 (F3) was assessed in *N. lugens* (Fig. 4). Comparison of these materials provides an opportunity to evaluate the extent to which eugenol enrichment contributes to insecticidal activity. Because eugenol has been widely recognized as the principal insecticidal constituent of clove oil, enhanced toxicity would be expected in fractions containing higher eugenol concentrations. Preliminary bioassays were conducted to identify, whereas advanced toxicity tests were used to establish dose-relationships and estimate LC_{50} and LC_{95} values, thereby providing a quantitative measure of insecticidal potency.

Preliminary Toxicity Test

Preliminary toxicity screening showed that both clove leaf oil and the eugenol-enriched fraction caused concentration- and time-dependent mortality in *N. lugens* **(**Fig. 4a**).** At 96 h, the highest concentration (0.020%) produced mortality of 63.33% and 53.33% for clove oil and the eugenol fraction, respectively, whereas 0.005% caused only limited mortality. Despite its lower eugenol content, clove oil consistently exhibited greater toxicity, suggesting additive or synergistic effects of minor constituents such as sesquiterpenes and other phenolics (Gad et al., 2023). The tested concentration range generated suitable mortality responses for subsequent probit analysis and lethal concentration estimation.

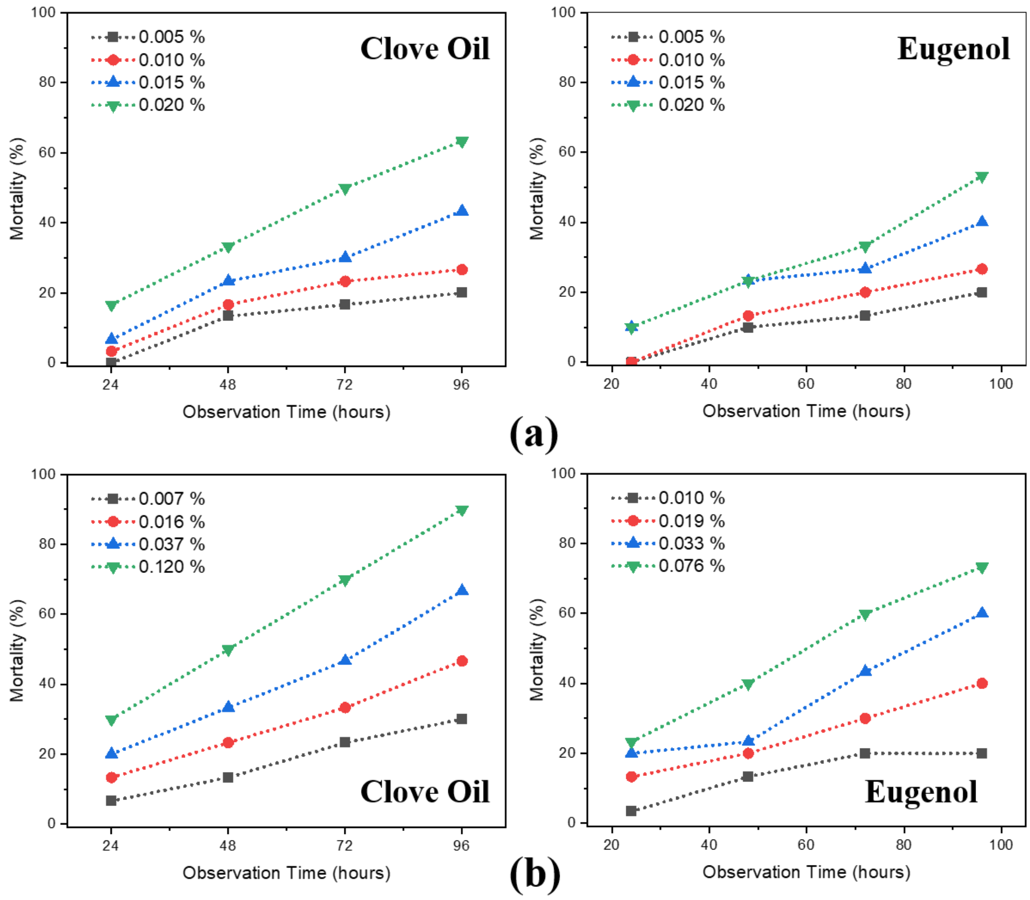

Figure 4. Toxicity evaluation of eugenol-based bioinsecticide against N. lugens: (a) preliminary toxicity test and (b) advanced toxicity test.

Table 1. Value of LC_{50} and LC_{95}

Insectiside	**a ± GBb**	**b ± GBb**	**LC_{50} (SK 50%) (%)**	**LC_{95} (SK 95%) (%)**
Clove Oil	-4.271 ± 1.165	1.929 ± 0.560	0.016 (123.40 – 298.19)	0.116 (492.67 – 24794.00)
Eugenol	4.664 ± 1.764	2.696 ± 0.942	0.019 (0.015 ± 0.041)	0.076 (0.037 ± 0.270)

Probit analysis revealed LC_{50} values of 0.016% and 0.019% for clove oil and the eugenol fraction, respectively (Table 1). These results indicate that clove oil exhibited slightly greater toxicity against *N. lugens* at median lethal concentrations. The corresponding LC_{95} values were 0.116% for clove oil and 0.076% for the eugenol fraction. The relatively low LC values obtained for both treatments demonstrate their potential as effective botanical insecticides. The LC_{50} estimates were subsequently used to establish the concentration range for advanced toxicity evaluation and dose-response analysis.

Advanced Toxicity Test

Definitive toxicity testing confirmed the strong insecticidal activity of both clove oil and the eugenol-enriched fraction against *N. lugens* (Fig. 4b). Mortality increased significantly with both concentration and exposure time, demonstrating sustained toxic effects throughout the observation period. Despite the higher purity of the eugenol fraction, clove oil consistently resulted in higher mortality across the tested concentration range. At concentrations approximating the LC_{50} values, clove oil caused 46.67% mortality after 96 h. At higher concentrations, clove oil achieved 90.00% mortality, whereas eugenol achieved 73.33% mortality. These results indicate that purification of eugenol did not necessarily translate into enhanced biological efficacy and suggest that minor constituents retained in the crude oil may contribute to toxicity through synergistic mechanisms, as commonly observed in essential oil-based insecticides.

The superior performance of clove oil relative to the purified eugenol fraction indicates that insecticidal activity may be influenced by the combined action of multiple constituents rather than eugenol alone. Minor compounds present in the crude oil may contribute to toxicity by enhancing cuticular penetration, increasing neurophysiological disruption, or interfering with detoxification pathways in insects (Dzięgelewska et al., 2025). In addition, the consistent concentration-dependent mortality observed in both treatments confirms the reliability of the concentration range established during preliminary screening. These results underscore the importance of phytochemical interactions in determining the biological efficacy of essential oil-based insecticides.

Comparison with Commercial Insecticides

Compared with the commercial insecticide fipronil, purified eugenol exhibited lower insecticidal efficacy but demonstrated several advantages associated with botanical insecticides. Fipronil (0.1%) produced complete mortality of *N. lugens* within 96 hours, reflecting its highly potent neurotoxic activity through inhibition of γ-aminobutyric acid (GABA)-gated chloride channels (Fig. 5). In contrast, eugenol produced substantial mortality and antifeedant effects while offering the potential benefits of lower environmental persistence and reduced ecological impact. These characteristics support its potential as a sustainable alternative for rice pest management.

Compared with commercial botanical insecticides such as neem-based formulations (*Azadirachta indica*), which have been reported to achieve mortality rates of approximately 93%, eugenol exhibited slightly lower lethality against *N. lugens*. Nevertheless, eugenol demonstrated substantial insecticidal and antifeedant activity, indicating its potential as an alternative botanical insecticide. These characteristics support its potential application in integrated pest management (IPM) programs as a complementary tool to reduce reliance on synthetic pesticides (Dzięgelewska et al., 2025).

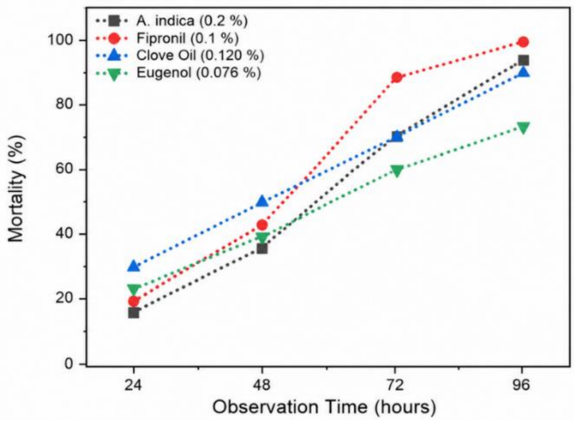

Figure 5. Comparison of brown planthopper mortality under treatments with a synthetic insecticide (active ingredient Fipronil 0.1%), a botanical insecticide (extract of Azadirachta indica 0.2%), and eugenol at the highest tested concentration.

Overall, the enrichment of eugenol through vacuum redistillation enhanced the chemical quality of clove leaf oil and enabled the production of a stable emulsified formulation with significant bioactivity against *N. lugens*. Although biological efficacy was influenced by interactions among multiple oil constituents, the purified fraction provided valuable insight into the role of eugenol as a major bioactive compound. These findings support the continued development of eugenol-based formulations as environmentally compatible alternatives for rice pest management (Soomro et al., 2025).

Antifeedant Activity and Agronomic Implications

Purified eugenol exhibited substantial antifeedant activity in addition to its insecticidal effects, reducing honeydew production by up to 60% relative to the control treatment (Fig. 6). Since honeydew excretion is widely used as an indirect measure of phloem feeding in planthoppers, this reduction indicates a marked suppression of feeding behavior. The antifeedant effect may be associated with disruptions in host acceptance, stylet activity, or feeding-regulation mechanisms, ultimately reducing nutrient acquisition. By comparison, crude clove leaf oil exhibited lower feeding inhibition, suggesting that eugenol enrichment enhanced antifeedant activity against *N. lugens*. Consistent with its lower penetration efficiency and lower neurophysiological interference.

As shown in Fig. 6a, insects treated with purified eugenol produced substantially less honeydew than those in the control group, indicating a reduction in phloem sap consumption. Because honeydew secretion is closely associated with feeding intensity in *N. lugens*, the decrease observed suggests effective suppression of feeding behavior by the treatment. This interpretation is further supported by the higher percentage of feeding inhibition shown in Fig.6b compared with crude clove leaf oil.

The antifeedant activity of eugenol has important implications for rice pest management because the damage it causes is primarily associated with sustained phloem feeding. Therefore, suppression of feeding activity may reduce nutrient depletion, minimize hopperburn symptoms, and decrease the transmission of plant pathogens. In addition to its insecticidal effects, eugenol exhibited strong feeding deterrence, indicating its potential as a multifunctional botanical pesticide (Lv et al., 2022). The present study further demonstrated that high-purity eugenol can be obtained from clove leaf oil through vacuum fractional distillation and subsequently formulated into a stable oil-in-water (O/W) bioinsecticide emulsion for *N. lugens* control.

In addition to its direct insecticidal activity, the formulated eugenol-based bioinsecticide exhibited ~~a~~ significant antifeedant effect, demonstrating a dual mode of action against *N. lugens*. The combination of lethal and sublethal effects may enhance pest suppression by reducing both insect survival and feeding-associated crop damage, thereby improving the overall effectiveness of pest management interventions. Beyond its biological efficacy, this study highlights a value-added approach to the utilization of clove leaf oil by enriching eugenol and incorporating it into stable bioinsecticide formulations. The findings contribute to the growing body of knowledge on plant-derived pest control agents and support the development of environmentally compatible alternatives to synthetic insecticides. By promoting the use of renewable botanical resources and reducing reliance on conventional chemical pesticides, the proposed formulation aligns with the objectives of sustainable agriculture and contributes to global sustainability initiatives, particularly SDG 2 (Zero Hunger), SDG 12 (Responsible Consumption and Production), and SDG 15 (Life on Land).

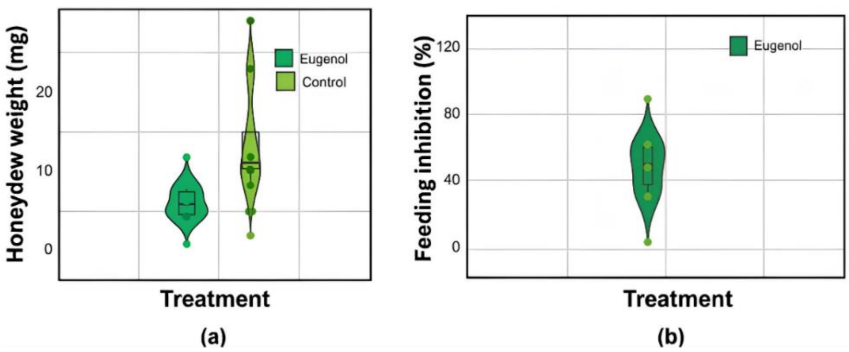

Figure 6. Antifeedant activity of eugenol against N. lugens at sublethal concentration (LC_{50}): (a) weight of honeydew excretion produced by treated insects and (b) mean feeding inhibition of brown planthopper.

CONCLUSION

Vacuum fractional distillation successfully enriched eugenol from clove leaf oil, increasing its concentration from 53.53% to 94.30%, and facilitated the development of a stable oil-in-water bioinsecticide formulation. The purified eugenol formulation exhibited significant insecticidal and antifeedant activities against *N. Lugens,* while crude clove oil and purified eugenol displayed LC_{50} values of 0.016% and 0.019%, respectively. Although crude clove oil produced higher mortality, purified eugenol induced stronger feeding inhibition, reducing feeding activity by up to 60%. These findings indicate that pest suppression can be achieved through both lethal and sublethal mechanisms and suggest that the biological efficacy of clove-derived products is influenced by both eugenol concentration and interactions among accompanying phytochemicals. Future studies should focus on field validation, formulation optimization, and assessment of long-term efficacy and environmental performance under diverse agricultural conditions.

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AUTHOR CONTRIBUTION STATEMENT

EC conceived the study, supervised the research, developed the methodology, interpreted the findings, and critically reviewed the manuscript. CM conducted the experiments, collected and analyzed the data, and prepared the original manuscript draft. SBMAA contributed to data interpretation, scientific discussion, and manuscript revision. SBWK assisted with experimental design, laboratory investigations, and data validation. DRI contributed biological assays, managed insect rearing, and interpreted entomological data. PPA supervised the bioassays, provided expertise in pest management, and contributed to data interpretation. RP participated in purification and formulation development, data analysis, visualization, and manuscript preparation. All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

AI-assisted technology was used exclusively for language editing and readability enhancement during the preparation of this manuscript. The authors independently conceived the study, conducted the research, analyzed and interpreted the data, and prepared the scientific content. All outputs generated with AI assistance were carefully reviewed and verified by the authors, who assume full responsibility for the final manuscript.

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