



Effect of Methanolic Extracts of Clove (*Syzygium aromaticum* L.) Leaves and Galangal (*Alpinia galanga* L.) Rhizome for the Management of Rice Brown Plant Hopper, *Nilaparvata lugens* (Stal.)

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ABSTRACT

Background: The utilization of plant-based pesticides derived from clove's leaf and galangal represents a potential alternative for pest control, offering relative environmental safety, particularly in the case of *Nilaparvata lugens* (Stal.) infestations on rice plants. The objective of this study was to determine the optimal formulation of a plant pesticide based on clove's leaf and galangal extracts for effective management of *N. lugens* on rice plants.

Methods: The research was conducted at the Green House of the Pest and Plant Disease Department, Faculty of Agriculture, Sam Ratulangi University, Manado. Six different concentrations of clove's leaf pesticide and galangal were used, each with four replications. These concentrations were 2 ml/L, 4 ml/L, 6 ml/L, 8 ml/L, 10 ml/L, and a control group. The parameters measured included nymph mortality at 12, 24, 48, and 72 hours after pesticide application. The application of the pesticide employed three methods: insect spraying, plant spraying, and insect-on-plant spraying.

Result: Our investigations indicated that insect spraying with clove leaf pesticide resulted in a mortality rate of 43.3% to 93.3%, while galangal-based pesticide caused a mortality rate of 13.3% to 80%. Plant spraying with clove's leaf pesticide led to a mortality rate of 6.7% to 46.7%, and galangal-based pesticide resulted in a mortality rate of 6.7% to 40.0%. Insect-on-plant spraying caused mortality rates of 46.7% to 86.7% for clove's leaf-based pesticide and 50% to 76.7% for galangal-based pesticide at 12 to 72 hours after application. The highest efficacy in terms of the quickest insect mortality occurred at a concentration of 10% using the insect spraying and insect-on-plant spraying methods, with mortality observed at 12 hours after application. The slowest response was observed at a concentration of 10% using the plant spraying method, with mortality occurring at 48 hours after application. The LT50 values for 10% clove's leaf and galangal applications were determined as 18.2 hours and 24.6 hours, respectively. Therefore, clove's leaf and galangal-based plant pesticides can serve as viable alternatives to synthetic pesticides for the management of *N. lugens*, a pest of rice plants.

Key words: Clove leaves, Galangal rhizome, Insecticidal properties, *Nilaparvata lugens*, Plant extract, Rice plants.

INTRODUCTION

Rice, a crucial staple crop for human sustenance, holds the third position globally among cereals, following corn and wheat. It serves as a primary source of carbohydrates for the majority of the world's population. Indonesia stands as the largest rice importer globally, accounting for 14% of the global rice market (Rainiyati and Aryanda, 2015; Sirappa, 2011). In North Sulawesi, rice productivity averages at 52.2 tons per hectare, which falls within the low productivity category when compared to the national average of 52.94 tons per hectare from 2011 to 2015. Several factors contribute to this low productivity, including the lack of planting high-quality and certified rice seeds by farmers, suboptimal cultivation systems and increased incidence of pests and diseases (Rozi *et al.*, 2018; Sianipar *et al.*, 2015). Particularly, the invasion of *Nilaparvata lugens*, a pest of rice plants, poses a significant challenge as it leads to substantial production losses, quality deterioration, disruption of production continuity and reduced income (Baehaki *et al.*, 2016; Senewe *et al.*, 2020).

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The control of *N. lugens* is crucial to ensure stable rice production in Indonesia (Rashid *et al.*, 2016). Essential oils derived from various plant sources, such as leaves, flowers, wood and seeds, hold promise as a means of controlling this pest (Devrnja *et al.*, 2022). Among the potential essential oils, clove leaves and galangal oils have garnered attention as plant-based pesticides (Abdullah *et al.*, 2015; Ikawati *et al.*, 2021; Sukhirun *et al.*, 2010). Clove oil contains several volatile compounds, including eugenol, eugenol acetate and methyl eugenol. Eugenol, the primary component of clove oil, is a colorless or pale-colored liquid that turns blackish-brown upon exposure to sunlight and possesses a distinct aroma (Nadeem *et al.*, 2023). While clove leaves oil typically contains 70% eugenol, the flower variant can reach up to 90% (Mahulette *et al.*, 2020). Galangal, a rhizome plant with medicinal properties, possess several active compounds which make it suitable for use as a plant pesticide. Galangal essential oil, characterized by its 1% content of methyl cinnamate, 20-30% cineole, eugenol, 1% camphor and sesquiterpenes, holds potential for use as an effective pesticide (Ruttanaphan *et al.*, 2020; Zhang *et al.*, 2021).

Medicinal plants and spices often contain active compounds suitable for use as raw materials in pesticide development. These compounds possess properties that can kill, repel, inhibit feeding and control plant diseases, making it essential to explore the potential of various medicinal plants and spices as plant-based pesticides (Cheraghi Niroumand *et al.*, 2016; Seepe *et al.*, 2021). Plants naturally produce secondary compounds that serve as defense mechanisms against invading organisms. Extracts of these chemical compounds have the potential to be used as more selective and less persistent plant-based pesticides compared to synthetic counterparts, thereby ensuring safety for farmers, users and surrounding environments (Tlak Gajger and Dar, 2021). Over 1,500 plants have been identified for their efficacy as sources of plant-based pesticides, primarily belonging to the Meliaceae, Annonaceae, Asteraceae, Piperaceae and Rutaceae families (Hernández-Lambrano *et al.*, 2014). However, the availability of plant-based pesticides with proven efficacy and safety remains limited. While farmers often employ various plant species empirically for pest control, the quality and safety of these products lack scientific accountability (Rv and Roy, 2014). Given the potential of several spice and medicinal plants in pest control, this study aims to investigate the effectiveness of clove leaves and galangal rhizome extracts in controlling *N. lugens* infestations on rice plants and determine the efficacy of a plant-based extract derived from these plants for the management of *N. lugens* pests.

MATERIALS AND METHODS

Preparation of clove leaves and galangal rhizomes extracts

The clove leaves and galangal rhizomes were dried in an oven at 35-40°C for 6-8 hours and subsequently ground

into a fine powder using a blender. The powdered materials were then soaked in methanol for 24 hours, filtered and repeatedly soaked until the extract reached a non-viscous consistency. The extract was then concentrated using a rotary evaporator at a temperature of 45-50°C until a concentrated extract was obtained.

Application of the extracts in test insects

The test insects, *N. lugens*, were obtained from a rice plant production center. The eggs and nymphs were reared and bred on rice plants placed in a confined environment. The nymphs used in this study were third instar nymphs from the second-generation imago. Two types of extracts, clove leaves and galangal rhizomes extracts, were employed at concentrations of 2 ml/L, 4 ml/L, 6 ml/L, 8 ml/L and 10 ml/L. Water was used as the control treatment.

The extracts' application in this study involved three distinct methods (Djau *et al.*, 2022). Firstly, "insect spraying" was conducted by placing ten insects in a flask and applying the equal appropriate quantity of the extracts to them, ensuring a light mist or coverage over the insects without causing excessive pooling in the flask. Subsequently, the treated insects were introduced to the confined area containing the rice plants. Secondly, "plant spraying" involved evenly distributing the extracts over the rice plants, after which the treated plants were placed in a confined area for observation. After allowing sufficient time for the extracts to dry due to wind exposure, ten insects were introduced to each treated plant. Lastly, the 'insect-on-plant spraying' method involved introducing thirty insects into a confined area where the rice plants were located. Following this, the extract was uniformly applied directly onto the insects while they were on the plants.

The research was conducted using each pesticide with 5 treatments, including a control group and replicated 4 times. The observed parameters included mortality and median lethal time (LT₅₀). Mortality was determined using the Abbot formula and assessed through the Chi-square (χ^2) method. Additionally, the LT₅₀ was calculated using Probit analysis. In this study, the notation "C" represents clove extracts, while "L" represents galangal rhizomes extracts. Additionally, the numerical designations are as follows: "1" corresponds to a concentration of 2 ml/L, "2" corresponds to 4 ml/L, "3" corresponds to 6 ml/L, "4" corresponds to 8 ml/L and "5" corresponds to 10 ml/L.

RESULTS AND DISCUSSION

Insect spraying

This research revealed a significant impact on the mortality of *N. lugens* nymphs at 12, 24, 48 and 72 hours after applying clove leaves and galangal rhizome extracts using the insect spraying method at various concentrations, as compared to the control treatment (Table 1). The Chi-square (χ^2) test conducted on the nymph mortality of *N. lugens* revealed significant effects from the application of clove's leaf and galangal extracts. Specifically, at 72 hours, the

mortality of *N. lugens* exhibited significant variation among the treatments. This indicates that the use of clove leaves extract was more effective in the insect spraying method compared to the application of galangal rhizome extract.

Plant spraying

The application of clove leaves and galangal rhizome extract using the plant spraying method did not show a significant difference in effect between treatment C1 and L1 when compared to the control treatment at 12 hours. However, treatments C2, C3, C4, C4, L2, L3, L4 and L5 demonstrated a significant difference from the control treatment (Table 2). Furthermore, the results of the Chi-square analysis indicated that the plant spraying method utilizing clove's leaf and galangal extract was not effective in controlling the pest *N. lugens*. Upon observing the results at 72 hours, it was found that treatments C5 and L5 resulted in 46.7% and 40.0% mortality, respectively. Consequently, to achieve a mortality rate of over 50% using the plant spraying method, higher concentrations of clove's leaf and galangal extract would be required.

Spraying insect on the plant method

The Chi-square test demonstrated a significant difference in the effects of the treatment and control at 12, 24, 48 and 72 hours when utilizing this method (Table 3). The application of clove's leaf and galangal extract through the plant spraying method proved to be highly effective in controlling *N. lugens*. Specifically, treatments C5 and L5 resulted in 66.7% and 53.3% mortality, respectively, after 24 hours.

Mortality rates

The Chi-square test revealed that treatment C5 resulted in higher mortality rates compared to the control at 12, 24, 48 and 72 hours (Fig 1). Similarly, the mortality of *N. lugens* in treatment L5 was higher than the control at 12, 24, 48 and 72 hours (Fig 2). These results suggest that higher concentrations of both extracts could expedite the mortality of *N. lugens*. This indicates that the concentration of clove leaves and galangal rhizome extracts had the ability to suppress or eliminate *N. lugens* as early as 12 hours after application.

Table 4 presents the LT_{50} values, or the time required to kill 50% of the *N. lugens* nymph population, when treated with extracts from clove leaves and galangal rhizome. The LT_{50} value for clove's leaf extract (labeled as C5) is 18.2 hours, whereas for galangal rhizome (labeled as L4), it is 24.6 hours. This suggests that the clove leaves extract is more effective at inducing mortality in a shorter timeframe compared to the galangal rhizome extract. Additionally, the upper and lower confidence limits for these LT_{50} values are also provided. Clove leaves has upper and lower limits of 23.7 hours and 12.6 hours, respectively, while for galangal rhizome, these limits are 30.2 hours and 19.9 hours. The narrower range of upper and lower limits for clove leaves suggests a more consistent and predictable effect, making it the preferred choice if rapid mortality is the objective. These findings offer a foundation for further investigations, perhaps focused on identifying the bioactive compounds

in each extract responsible for the observed effects and exploring potential synergistic interactions between them.

The plant extracts have demonstrated their capability to effectively control *N. lugens*, as indicated by q value analysis with similar percent mortality or >50% efficacy in insect spraying and insect-on-plant spraying methods, using either clove leaves extract (93.3%) or galangal rhizome extract (80.0%). However, the plant spraying method was not effective in controlling *N. lugens*. Throughout the 72-hour observation period, the mortality rates steadily increased in all treatments, albeit with slight increments. This suggests the presence of active

Table 1: The mortality rates of *N. lugens* following the application of clove leaves and galangal rhizome extracts, administered using the insect spraying method.

Treatments	Mortality (%) at time (hours)			
	12	24	48	72
C1	0	6.7*	30.0*	43.3*
C2	0	20.0*	40.0*	56.7*
C3	0	33.3*	53.3*	66.7*
C4	0	56.7*	66.7*	80.0*
C5	3.3	73.3	80.0	93.3*
L1	0	0	6.7*	13.3*
L2	0	3.3	20.0*	33.3*
L3	3.3	10.0*	40.0*	50.0*
L4	6.7	23.3*	50.0*	63.3*
L5	6.7	40.0*	60.0*	80.0
Control	0	0	0	0

The asterisks indicate a significant difference ($p < 0.05$) in the mortality rates at those specific time points compared to the mortality rate of the control group.

Table 2: The mortality rates of *N. lugens* following the application of clove leaves and galangal rhizome extracts, administered using the plant spraying method.

Treatments	Mortality (%) at time (hour)			
	12	24	48	72
C1	0	0	0	6.7*
C2	0	0	13.3*	26.7*
C3	0	3.3	20.0*	40.0*
C4	0	6.7*	30.0*	43.3*
C5	6.7*	20.0*	40.0*	46.7*
L1	0	0	3.3	6.7*
L2	0	0	10.0*	13.3*
L3	0	10.0*	16.7*	30.0*
L4	3.3	10.0*	20.0*	36.7*
L5	10.0*	13.3*	23.3*	40.0*
Control	0	0	0	0

The asterisks indicate a significant difference ($p < 0.05$) in the mortality rates at those specific time points compared to the mortality rate of the control group.

Table 3: The mortality rates of *N. lugens* following the application of clove leaves and galangal rhizome extracts, using the insect-on-plant spraying method.

Treatment	Mortality (%) at time (hour)			
	12	24	48	72
C1	0	26.7*	40.0*	46.7*
C2	0	26.7*	50.0*	56.7*
C3	3.3	40.0*	56.7*	73.3*
C4	6.7*	43.3*	73.3*	83.4*
C5	6.7	66.7	80.0	86.7
L1	3.3	6.7*	36.7*	50.0*
L2	3.3	6.7*	40.0*	56.7*
L3	10.0*	20.0*	56.7*	70.0*
L4	10.0*	50.0*	70.0*	76.7*
L5	10.0*	53.3	76.7	76.7
Control	0	0	0	0

The asterisks indicate a significant difference ($p < 0.05$) in the mortality rates at those specific time points compared to the mortality rate of the control group.

compounds in the pesticide responsible for the insecticidal effects (Hastuti *et al.*, 2015; Hikal *et al.*, 2017).

Utilizing clove leaves and galangal rhizome extracts is a more environmentally friendly approach for *N. lugens* control, given their biodegradable and low mammalian toxicity characteristics (Bezabih *et al.*, 2022; Ghosh *et al.*, 2012). The high mortality observed in *N. lugens* may be attributed to the presence of intoxicating and repellent active compounds, effectively eliminating the pests (Poonsri *et al.*, 2019; Ruttanaphan *et al.*, 2020). Galangal rhizome extract, specifically, contains nitric oxide inhibitors, which could reduce the body's immune response against foreign materials, possibly leading to the mortality of *N. lugens* through a decline in the immune system (Abdullah *et al.*, 2015; Ahlina *et al.*, 2020). Galangal rhizome extract contains terpenoids, alkaloids and phenols that act as insecticides and fungicides (Charles *et al.*, 1992). The substances present in galangal rhizome inhibit the activity of acetylcholinesterase, an enzyme vital for nerve impulse transmission. This disruption in nerve activity leads to

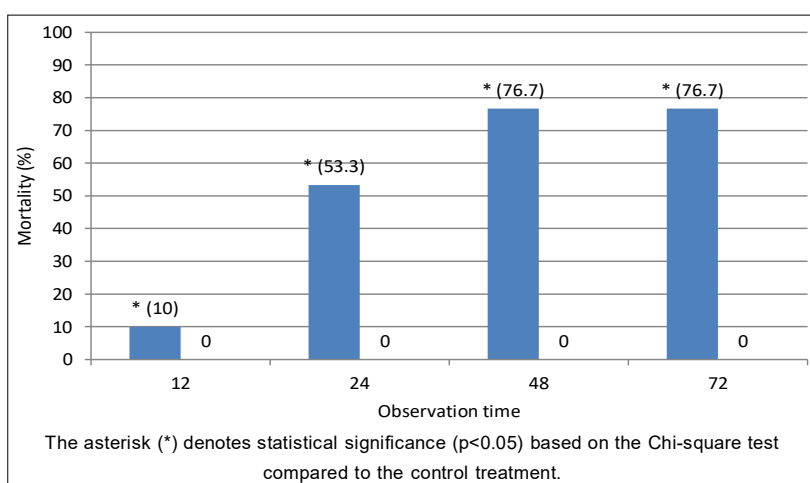
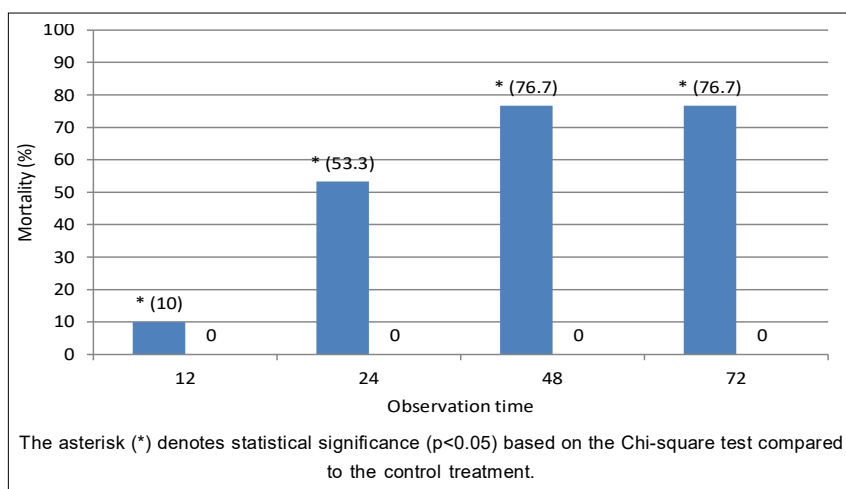
**Fig 1:** The mortality rates of *N. lugens* in the clove leaves extract treatment.**Fig 2:** The mortality rates of *N. lugens* in the galangal rhizome extract treatment.

Table 4: Median lethal time (LT₅₀) values of *N. lugens* nymphs treated with clove leaves and galangal rhizome extracts.

	LT ₅₀ value (hour)	Upper limit (hour)	Lower limit (hour)
Clove's leaf (C5)	18.2	12.6	23.7
Galangal (L4)	24.6	19.9	30.2

incoordination, weakness, staggering and eventual mortality (Guo *et al.*, 2010).

The use of higher concentrations of plant extracts results in more toxins reaching the cuticle of the insects, inhibiting their growth and causing higher mortality (Akbar *et al.*, 2022; Zaka *et al.*, 2019). Additionally, in high concentrations, clove oil can anesthetize and intoxicate *N. lugens*, leading to reduced movement, loss of responsiveness, decreased appetite and eventual death (Mardiningsih and Rohimatun, 2021). Several factors influence the time it takes for the plant extract to kill the insects, such as temperature, humidity and sprayed concentration, which affect the delivery of active compounds to the target site (Ferraz *et al.*, 2022; Tembo *et al.*, 2018).

The efficacy of plant extracts as plant pesticides is also influenced by the insect's developmental stage when the extract is applied (Poderoso *et al.*, 2016). Different stages of insect development exhibit various skin change patterns, impacting the effectiveness of the applied extract concentration (Nisar *et al.*, 2021). Young insects or nymphs have less active movement than adults, increasing the likelihood of active compounds adhering to their integument (Nisar *et al.*, 2021). The thin and soft integument of nymphs facilitates the entry of active compounds into their bodies (Poderoso *et al.*, 2016).

Our finding showed that clove leaves and galangal rhizome extracts offer a potent and eco-friendly alternative to synthetic pesticides for the control of *N. lugens*. These extracts show promising results, with high levels of efficacy in insect spraying methods, largely due to their active compounds that interfere with the insect's physiological and neurological functions. However, it is crucial to note that the application method and insect developmental stage significantly influence the effectiveness of these plant-based solutions. Future research should explore optimizing application techniques, the impact of environmental conditions and identifying the most vulnerable stages of insect development to maximize efficacy.

CONCLUSION

Clove leaves and galangal rhizome extracts demonstrated significant effectiveness in controlling the pest *N. lugens*. At a concentration of 10 ml/L, the clove leaves and galangal rhizome extracts resulted in mortality rates of 93.3% and 76.7%, respectively, at 72 hours after application. The insect-on-plant spraying method and the insect spraying method were found to be the most effective spraying techniques.

Additionally, the LT₅₀ was recorded as 18.2 hours for the treatment with clove's leaf extract and 24.6 hours for the treatment with galangal rhizome extract. The study suggests that clove leaves and galangal rhizome extracts can serve as effective and eco-friendly plant-based insecticides for controlling *N. lugens*. Further research is needed to optimize their application methods and concentrations, assess their impact on non-target organisms and the environment and evaluate potential resistance development. Integrating these extracts into integrated pest management practices could promote sustainable agriculture.

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Conflict of interest

The authors have no conflicts of interest to declare.

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