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Effectiveness Test of Botanical Pesticide Formulation of Combination of Lime Leaves and Lemongrass Against Aphids (*Aphis spp.*) on Katokkon Chili Plants *in-Vitro*

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Abstract

Aphids (*Aphis spp.*) are one of the main pests that damage Katokkon chilies by sucking plant sap and transmitting viruses. Excessive use of chemical pesticides can lead to resistance and environmental problems; therefore, environmentally friendly alternatives are needed. This study aims to determine the effectiveness of a botanical pesticide formulation derived from lime (*Citrus spp.*) leaves and citronella (*Cymbopogon nardus*) against *Aphis spp.* Mortality *in vitro*. This experiment used a completely randomized design with 7 treatment levels (R1-R5, KP, KN) and 5 replications. Observed parameters included physicochemical stability of the formulation (pH, density and viscosity), pest mortality from 4-72 hours and toxicity based on probit analysis (LT50). Results showed that formulations were stable in storage for 14 days, with the smallest changes in pH, density and viscosity. The highest mortality was found in R4 (83.33%), followed by R3 (70.83%) and R2 (62.50%), while solvent control reached only 2.50%. Probit analysis revealed that R4 gave the shortest LT50 value (8.70 hours), followed by R3 (24.93 hours) and R2 (35.61 hours). Based on these results, a combination of lime and citronella, with a ratio of 25%:75% (R4), is the most effective formulation as a botanical pesticide against *Aphis spp.*

Keywords: *Aphis spp.*, Botanical Pesticides, Lime Leaves, Lemongrass, LT50, Mortality

1. Introduction

Chili pepper (*Capsicum annum* L.) is widely cultivated as a vegetable in Indonesia (Daryanto et al. 2020) and is the fourth most important crop worldwide in terms of economic value (Maugio et al. 2021). One of the large chilis is Katokkon chili, a superior local commodity from Toraja. This chili has very high economic value, with market prices reaching Rp 40,000 - Rp 60,000 per kilogram, which is higher than the average national chili price, which could reach Rp 100,000 per kilogram during peak season (Driyunitha, 2025). Superior quality of Katokkon chili is its special aroma; it is very spicy, with a heat level of 30,000 - 50,000 SHU, and it is shaped like a miniature bell pepper (Kasmianti, 2021). As an endemic variety, Katokkon chili has great potential, but farmers' productivity is still low. Production limitations are often caused by OPT attacks involving aphid pests (*Aphis spp.*) (Kaloli, 2021; Syaifudin, 2023). Aphids cause damage by

siphoning fluids from plants and by spreading virus diseases such as Cucumber Mosaic Virus (Manzoukas & Lagogiannis, 2019). An infected plant will exhibit wilting symptoms, leaf shrinkage and a decrease in growth rate (Jaya et al. 2024). Besides, aphids excrete a sweet liquid resembling yellow honey that attracts ants to feed on it (Minanda & Bambang, 2022). A high number of aphids can cause chlorosis of leaves, leaf fall, and the production of small fruit (Franco 2019). Color variants of aphids (*Aphis spp.*) include yellow, cream, green, and brown (Gamboa et al. 2021), and they infest chili throughout its growing season (Farhan 2021). Losses from aphid attacks on chili plants can reach 10-30% and 40% during the dry season (Ma'aruf et al. 2024). Because of the high intensity of the aphid pest attack, control is necessary. Control for aphid pests still relies on synthetic pesticides, which can pose risks of insect resistance, environmental pollution, hazardous residues in crops and high cost, as well as killing

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beneficial insects and even causing the proliferation of insect pest populations (Puspasari et al. 2024, Ghosh 2020). The use of synthetic chemical pesticides, particularly on horticultural crops, in Indonesia is extremely high. Hence, botanical pesticides should be developed as an alternative using natural, environmentally friendly and sustainable resources.

Botanical pesticides are pesticides whose active ingredients are derived from plant extracts. They protect plants from pests and diseases and are environmentally friendly. This is because these compounds can act as repellents, exterminators, appetite suppressants, and cause discomfort for pests. The active ingredients in plant extracts have been proven to replace synthetic chemical pesticides. Botanical pesticides can be a potential solution because they are biodegradable, low-toxic to non-target organisms, and can be produced independently by farmers.

Toraja has natural resources with potential as bioactive botanical pesticides, including lime leaves (*Citrus spp.*) and citronella (*Cymbopogon nardus*), which have not been fully utilized. Lime leaves contain *limonene*, *linalool*, and *citronellol acetate*. (Mariana et al. 2024). Meanwhile, lemongrass has a distinctive, strong aroma due to its essential oil content, which can be extracted from its leaves and stems. According to Mumba & Rante (2020), citronella oil contains 35.97% citronellal, 17.28% geraniol, 10.03% citronellol, 4.44% geranyl acetate, 4.38% elemol, 3.98% limonene, and 3.51% citronellyl acetate. Furthermore, citronella leaves contain phytochemicals such as flavonoids, saponins, tannins, steroids, phenolics, and polyphenols, which can act as insecticides (Najma et al. 2023). The bioactive compounds found in these two types of plants can be insecticidal and insect-repellent. Citronellal and geraniol compounds enter the insect's body as contact poisons that target the nervous system, disrupting larval activity and causing dehydration in pests (Mariana et al. 2024). Citronellal and limonene act as stomach poisons that disrupt the digestive systems of pests (Nopriansyah 2023). The combination of these two extracts is expected to produce a synergistic effect, enhancing the effectiveness of the botanical pesticide. This innovation not only reduces reliance on synthetic chemical pesticides but also supports the government's sustainable agriculture program.

Essential oil-based botanical pesticides, such as lime leaf extract and citronella, offer a safer alternative with diverse mechanisms of action (repellent, anti-inflammatory, and toxic). The combination of the two ingredients is expected to produce a synergistic effect, increasing control effectiveness. Botanical pesticides are a safer alternative, readily biodegradable, and have a low risk of residue. Lime leaves contain limonene and linalool, while citronella contains citronellal, citral, and geraniol, which have insecticidal potential. Therefore, the combination is predicted to produce a synergistic effect.

2. Material and Methods

This research was conducted from October 2025 to March 2026 at the Agrotechnology Laboratory and Experimental Garden of the Faculty of Agriculture, Toraja Christian University of Indonesia. 2.95730. S, 119.89589. E, 856 masl. The research consisted of laboratory testing and observation of the effectiveness of treatments on target organisms.

2.1. Research Flowchart

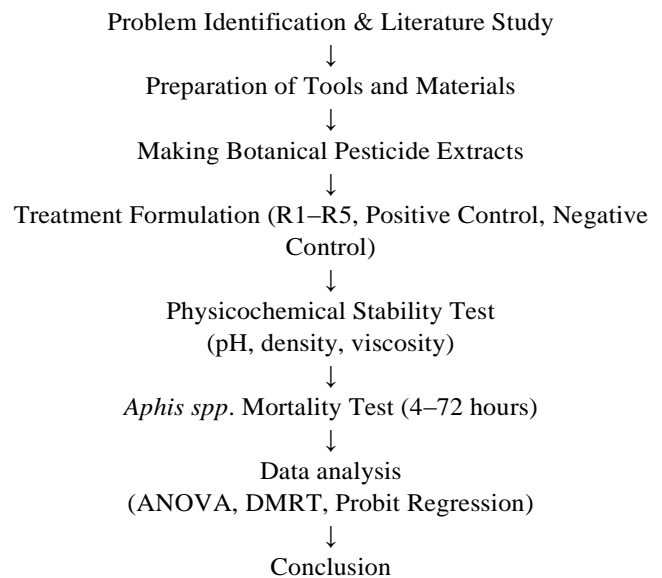


Figure 1. Flowchart Study

Laboratory research used a Completely Randomized Design (CRD) with seven treatments, namely:

R1 = 100% lime leaf extract

R2 = 75% lime leaves: 25% lemongrass

R3 = 50% lime leaves: 50% citronella

R4 = 25% lime leaves: 75% lemongrass

R5 = 100% citronella

KP = positive control (chemical pesticide)

KN = negative control (water)

Each treatment was repeated five times to obtain 35 experimental units. The main parameters observed included the mortality of test organisms at 4, 8, 12, 24, 48, and 72 hours after treatment application. In addition, measurements of the extract's physicochemical characteristics, including pH, density, and viscosity, were performed on days 0 and 14 of storage.

Observation data were analyzed using one-way analysis of variance (ANOVA). If the treatment showed a significant effect, the Duncan Multiple Range Test (DMRT) was used at a 5% confidence level. Probit regression was performed to estimate the LT50 based on the relationship between the mortality probit and the log10

of the observation time.

3. Results and Discussion

3.1. Physiochemical Changes (pH, Density, and Viscosity)

Physical stability is an important parameter in assessing the quality of botanical pesticide formulations because it determines the consistency of performance during storage and application. Observed parameters included pH, density, and viscosity during 14 days of storage at room temperature. Based on the study results, both formulations—lime leaf and lemongrass—exhibited changes in physical properties, but these were within normal limits and showed no signs of severe degradation. The increase in pH in both formulations was due to mild oxidation of organic components, such as terpenoids and phenolics. This is consistent with the stability characteristics of botanical extracts, where pH changes

commonly occur during storage due to natural oxidative reactions (Siagian, 2023; Irawati, 2018). The pH range of 5–6.5 is still relatively stable, does not trigger microbial growth, and does not damage the main bioactive components, such as limonene and citronellal, found in lime leaves and lemongrass (Mariana et al., 2024; Najmah et al., 2023).

The formulation density increased very slightly (<0.005 g/mL) from day 0 to day 14. An increase in density within this range indicates that the colloidal system in the formulation remains stable and no phase separation occurs. A slight increase in density in plant extracts is generally associated with small amounts of solvent evaporation or strengthening of intermolecular interactions in the solution system (Irawati, 2018). Density stability is important to ensure consistent spray dosage and even droplet distribution on small pests such as *Aphis spp.*

Table 1. Physical Stability of Botanical Pesticides During 14 Days of Storage at Room Temperature

Botanical Pesticides	Parameter	Day 0	Day 14
Lime leaves	pH	5.00	6.00
	Density (g/mL)	0.9606	0.9652
	Viscosity (cP)	69.16	77.22
Lemongrass	pH	5.50	6.50
	Density (g/mL)	0.9517	0.9540
	Viscosity (cP)	63.95	72.50

The viscosity of the formulation increased from 69.16 cP to 77.22 cP in the lime leaf extract and from 63.95 cP to 72.50 cP in the citronella grass extract. This moderate increase in viscosity is still within the stable limits for botanical pesticide formulations. The increase in viscosity may be influenced by hydrogen bonding between phytochemical components, such as flavonoids, saponins, and essential oils, as reported in studies on the physical characteristics of botanical extracts (Najmah et al., 2023; Mariana et al., 2024). Increasing viscosity can even be advantageous because it enhances the solution's adhesion to leaf surfaces and insect bodies, extending contact time and increasing toxic effectiveness. Overall, changes in pH,

density, and viscosity in this study indicate that the botanical pesticide formulation made from lime leaves and lemongrass remained stable for 14 days of storage and did not exhibit significant physical degradation.

3.2. Effectiveness of Formulation on Mortality of Aphids (*Aphis spp.*)

Biological testing results showed that the lime leaf and citronella grass formulation was significantly effective in controlling aphids. Mortality increased with increasing treatment concentration, with treatment R4 yielding the best results after 72 hours.

Table 2. Aphid Mortality 72 Hours After Treatment Application

Treatment	Mortality (%) (Mean ± SD)	Group
KN (chemical pesticide)	100.00 ± 0.00 a	a
R4 (25% lime leaves: 75% lemongrass)	83.33 ± 5.71 b	b
R3 (50 % lime leaves: 50% citronella)	70.83 ± 4.66 c	b
R2 (7.5 % lime leaves: 25% lemongrass)	62.50 ± 9.32 d	c
R1 (100 % lime leaves)	50.00 ± 8.72 e	d
R5 (100% citronella)	47.92 ± 10.42 f	d
KP (Water)	2.50 ± 3.42 g	e

Information: The average value in the column followed by the same letter is not significantly different at the $\alpha = 0.05$ level based on Duncan's Multiple Range Test (DMRT)

Aphid mortality in treatments R4 (83.33%) and R3 (70.83%) indicates that the combination of active ingredients in both formulations is capable of producing a

strong toxic effect. Lime leaves and citronella contain bioactive compounds, including limonene, citral, geraniol, and citronellal, which are insecticidal and repellent

(Mariana et al., 2024; Najmah et al., 2023; Mumba & Rante, 2020). These terpenoid compounds can act by inhibiting acetylcholinesterase (AChE) activity or by disrupting the insect's respiratory system, thereby reducing feeding activity and causing death. This finding is in line with the report of Jaya et al. (2024), which shows that lime leaf extract is effective in increasing the mortality of *Aphis spp.* on chili plants, as well as research by Sari et al. (2024), which proves that the combination of citronella with other plant materials increases efficacy against *Aphis gossypii*. In other words, the combination of the two extracts does provide a synergistic effect in controlling aphids.

Treatments R1 and R5 resulted in lower mortality (50.00% and 47.92%), indicating that the active ingredient concentrations in both treatments were below the effective threshold or worked less than optimally. The positive

control using water (KP) showed a mortality of only 2.50%, indicating that the mortality was not due to the activity of plant compounds. The combination of lime leaves and lemongrass provides an insecticidal effect through several mechanisms, namely: *Neurotoxic* - essential oils such as limonene and citral have been shown to affect the nervous system of insects (Nopriansyah, 2023), *Antifeedant* - phenolic components can reduce feeding intensity, causing insects to weaken and die (Farhan, 2021), *Cuticle damage* - essential oils can dissolve the wax layer of the insect's body, causing dehydration (Najmah et al., 2023), *Fumigant effect* - volatile characters allow compounds to enter the spiracles and interfere with respiration (Mumba & Rante, 2020). This synergy of mechanisms explains why the mortality rate in the high-concentration treatments (R3 and R4) was much higher than in other treatments.

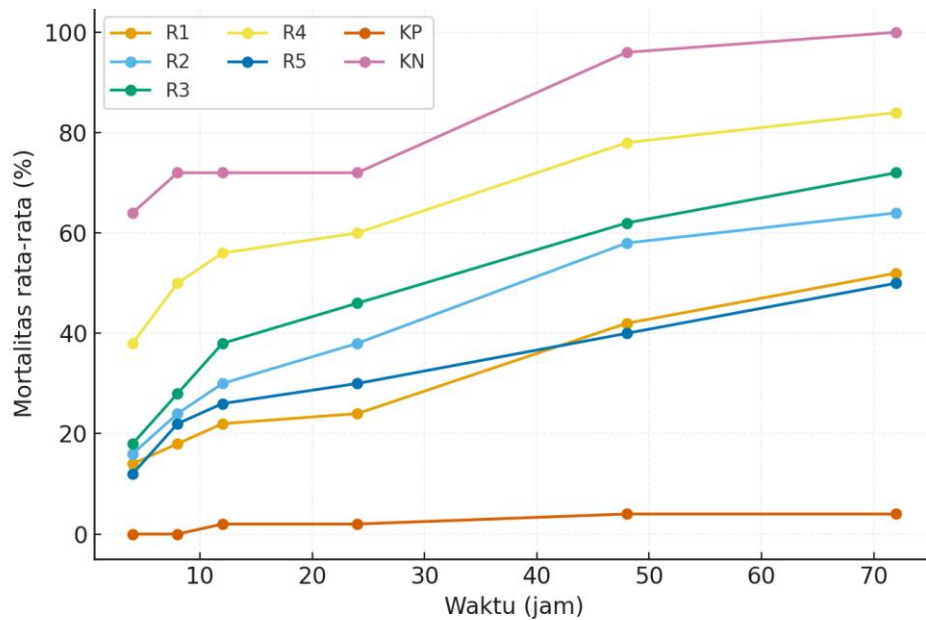


Figure 2. Mortality trend of aphid pests in 4-72 hours

Based on the 4–72-hour mortality graph, all treatments showed a gradual increase in mortality, but with varying effectiveness. Treatments R4 and R3 showed the highest rate of increase, indicating that the active ingredient concentrations in both formulations had stronger insecticidal activity against *Aphis spp.* Conversely, R1 and

R5 showed a slower increase, indicating lower effectiveness. The positive control (KP) did not cause significant mortality, further confirming that the toxic effect originated from the active components of the botanical pesticides.

Table 3. Toxicity Parameters and Results of Probit Regression Analysis on Aphids

Treatment	Slope	Intercept	R ²	LT50 (hours)
R1	0.8787	-1.7032	0.925	86.74
R2	1.0910	-1.6927	0.985	35.61
R3	1.1573	-1.6165	0.991	24.93
R4	1.0000	-0.9396	0.963	8.70
R5	0.8442	-1.6170	0.969	82.32
KN	2.2360	-1.5334	0.673	4.85

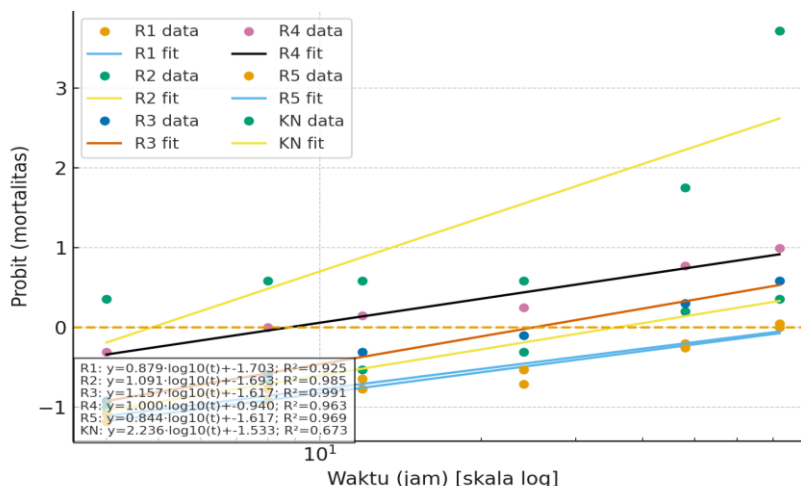


Figure 3. Probit regression graph

Table 3 shows that each treatment had different slope, intercept, and LT50 values, reflecting variations in the speed and toxic effectiveness of the formulations against *Aphis spp.* Treatments R3 and R2 had the highest slopes (1.1573 and 1.0910), indicating a faster, relatively homogeneous mortality response throughout the exposure period. This kind of linear response is a common characteristic of essential oil-based plant compounds with high volatility and penetration (Mariana et al., 2024; Najmah et al., 2023).^{16,18} This consistency is evident in the combined probit regression graph, where the R3 and R2 regression lines appear steeper than those of the other treatments. This indicates an increase in aphid sensitivity to exposure to the active compounds in both formulations. This phenomenon is also supported by the report by Jaya et al. (2024)⁸, which found that citrus leaf extract can increase the toxic response of *Aphis spp.*, progressively with increasing exposure time.

Although R3 and R2 have steeper slopes, the formulation with the fastest toxic action speed is still R4, with the lowest LT50 of 8.70 hours. This speed is related to the high content of volatile compounds in citronella, such as citronellal, geraniol, and citral, which are known to provide a faster knockdown effect on sucking insects (Mumba & Rante, 2020; Najmah et al., 2023)^{17,18}. The ability to penetrate the spiracles and the neurotoxic effects of terpenoids accelerate insect death, so the LT50 of R4 is lower than that of the other formulations. In contrast, treatments R1 and R5 had lower slopes (0.8787 and 0.8442), indicating slower, less homogeneous mortality responses. This is consistent with their higher LT50 values (86.74 and 82.32 hours), indicating that both formulations required longer times to reach 50% mortality. This is understandable because R1 and R5 are single extracts without a combination, thus preventing potential synergy between active compounds. Studies by Ahmad (2024)^{and} Sari et al. (2024)^{also} showed that combining two types of plant materials led to a faster mortality response than a

single extract. The negative control using chemical pesticides (KN) had the highest slope (2.2360) and the fastest LT50 (4.85 hours). This reflects the mechanism of action of synthetic chemical pesticides, which are fast and aggressive in inhibiting the pest's nervous system. However, the lower R² value (0.673) indicates variability in response over time, a common characteristic of chemical contact insecticides with instant knockdown effects.

Overall, the relationship between the slope, the position of the regression line, and the LT50 value in this study indicates that the formulation with the highest toxicity potential is R4, followed by R3 and R2. All three formulations have optimal concentrations of active compounds with strong insecticidal activity. This finding is consistent with the studies by Sari et al. (2024)²², Najmah et al. (2023)¹⁸, and Jaya et al. (2024)⁸, which emphasized that the combination of essential oil-based botanical ingredients resulted in a significant increase in toxicity and a decrease in LT50 values. However, this study was still conducted under controlled laboratory conditions, so it does not reflect the variability of environmental factors such as temperature, humidity, light intensity, and rainfall that can affect the stability and effectiveness of botanical pesticides in the field. Furthermore, this study only focused on *Aphis spp.* without evaluating potential side effects on non-target organisms such as predators and parasitoids. Therefore, actual effectiveness in open agroecosystems still needs to be verified.

4. Conclusion

The formulations of the botanical pesticides were stable in terms of pH, density and viscosity, with minimal fluctuations over 14 days, indicating they were fit for use. The formulations varied in their killing efficacy; formulation R4 demonstrated the highest mortality, which increased up to 72 h, followed by R3 and R2. Formulations R1 and R5 had poor mortality; none in the solvent control. The LT50 values indicated that formulation R4 had the

fastest killing rate (8.70 h), followed by R3 and R2. Formulations R1 and R5 recorded slower kill times. The trend in LT50 values correlated with a probit regression

and confirmed that formulation R4 was the best for killing *Aphis spp.*

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