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Efficacy of Thyme and Eucalyptus Essential Oils as Biopesticides for *Macrosiphum euphorbiae* Control

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ABSTRACT

Plant essential oils, with their rapid breakdown, low toxicity, and environmental safety, serve as a promising natural alternative for pest control. This study investigates the insecticidal potential of *Eucalyptus* and *Zataria* essential oils against *Macrosiphum euphorbiae*. The oils were extracted by water distillation using a Clevenger device, and bioassay tests were performed on adult specimens at different concentrations. Probit analysis determined the LC50 and LC90 values for eucalyptus oil at 4699 ppm and 37106 ppm, respectively, while for thyme oil, these values were 11944 ppm and 45620 ppm. These findings indicate that eucalyptus oil has a stronger toxic effect and higher mortality rate than thyme oil. The peak mortality rate occurred at 20,000 ppm for eucalyptus and 24,000 ppm for thyme. Since the LD50 values for both essential oils are lower than synthetic insecticides, and resistance to chemical treatments continues to grow, these plant-based alternatives could serve as effective substitutes for conventional pest control solutions.

Keywords: Essential oils, Thyme, Insecticidal effects, Eucalyptus

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Introduction

The increasing reliance on chemical insecticides to mitigate pest damage has led to significant risks for both human health and the environment. Excessive application of these synthetic compounds has resulted in the development of resistance among pest insects, contamination of food sources, toxicity to mammals, and harm to environmental degradation and non-target organisms [1, 2]. Recognizing these hazards, there has been a growing interest in alternative pest control strategies that maintain effectiveness while minimizing ecological and health risks. Among these alternatives, plant-derived compounds have gained attention due to their natural composition and safety profile for both humans and the environment.

Potato is the most crucial crop worldwide, providing essential nutrients such as proteins, carbohydrates, vitamins, and energy-generating compounds. It holds a strategic position alongside staple grains like wheat, barley, and rice. Acknowledging its significance, the World Food Organization designated 2008 as the “Year of the Potato” [3, 4]. Notably, a substantial portion of global potato production occurs in developing nations, particularly across Asia.

Potato crops are vulnerable to numerous pests, with aphids being among the most destructive. These pests weaken plants by extracting sap, leading to reduced crop yield and quality. Additionally, aphids serve as vectors for multiple harmful potato viruses, with at least ten major viral diseases being transmitted through their activity. High aphid populations can severely impact plant health and agricultural output, increasing production costs and economic losses [5, 6].

At present, chemical insecticides remain the primary method for controlling potato aphids. However, the indiscriminate use of these chemicals has contributed to widespread resistance, making pest management increasingly challenging. Furthermore, their adverse effects on beneficial organisms and the surrounding environment have raised concerns. Due to the limitations of synthetic insecticides, there has been a shift toward plant-based insecticidal compounds as a sustainable alternative for aphid management. These botanical solutions decompose rapidly, leaving no harmful residues in soil or water, and pose minimal risk to non-target insect populations [7-9].

This study aims to evaluate the insecticidal properties of essential oils from two medicinal plants and assess their potential for pest control while reducing the environmental risks associated with conventional chemical insecticides.

Materials and Methods

For this study, the leaves of the selected plants were gathered during the flowering stage of eucalyptus and thyme. To prepare them for essential oil extraction, the leaves were air-dried under shade at a temperature of room with adequate ventilation. Once dried, the plant material underwent essential oil extraction using the Clevenger apparatus through the water distillation method **(Figure 1)**.

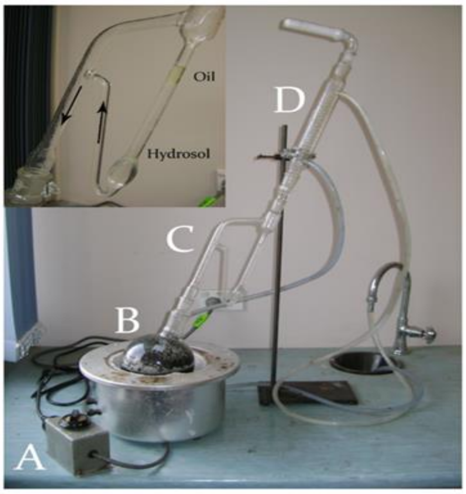

Figure 1. Clevenger-type water distillation system: (a) power regulator, (b) heating mantle with a round-bottom flask containing water and aromatic plant leaves, (c) Clevenger apparatus, which allows hydrosol to return to the still while retaining the essential oil phase—applicable for oils less dense than water that float on the surface, and (d) condenser.

Collection and rearing of potato aphid colony

Aphid collection commenced as soon as the pest was observed on potato plants in the study field. The aphids were initially transferred onto freshly cut potato leaves sourced from the same field to establish a stable population for further experimentation. These leaflets were put in Petri dishes containing moistened filter paper and cotton to maintain humidity. The prepared Petri dishes were then relocated to a controlled growth chamber. Every two days, the existing leaves were replaced with fresh ones. Before initiating the main experiments, the aphid population was transferred onto potato plants cultivated under greenhouse conditions.

Host plant cultivation

For this experiment, ten plastic pots, each with a 50 cm diameter and a height of 15 cm, were prepared by filling them with a soil mixture composed of two-thirds agricultural soil and one-third sand. A single tuber of the Sprite potato cultivar was planted in each pot. The plants were cultivated in greenhouse conditions, with irrigation carried out every 4 days, and a single application of urea fertilizer was added to the soil during the test period. Once the plants reached the five- to six-leaf stage, specific leaflets were collected for testing. The 2nd and 3rd compound leaves from the top of each plant were carefully cut and used in the test. To prevent aphid escape, transparent plastic arches were installed over the pots, secured to a silk net fabric covering the top.

Aphid synchronization

To conduct biometric assessments, it was essential to use aphids of the same developmental stage. The leaf disc method was employed for this purpose [10]. Adult aphids were gently removed from infected potato plants using a fine brush and transferred onto healthy potato leaf discs, which were put inside Petri dishes with a diameter of 8 cm. To maintain proper humidity, moistened cotton soaked in distilled water was put at the bottom of the Petri dishes, followed by a 9 cm filter paper layer to ensure a stable surface for the aphids. The filter paper also prevented the insects' legs from getting stuck during movement. A freshly cut potato leaf was positioned on the filter paper with its underside facing upward. Each leaf disc accommodated approximately 10–20 adult aphids.

A ventilation hole, 2 cm in diameter, was made in the Petri dish lid, covered with fine mesh fabric. The aphids were left undisturbed for 24 hours to facilitate nymph production. After this period, aphids who were adults were removed, leaving behind nymphs of uniform age. These nymphs were then reared under identical conditions until they matured into adults after 7–8 days. The rearing process was conducted in an incubator set to 25 ± 1°C, with a relative humidity of 65 ± 5% and a 16-hour light/8-hour dark photoperiod [11, 12].

Determination of essential oil concentrations

To establish appropriate concentrations of essential oils that would induce mortality in potato aphids, a series of preliminary experiments were conducted. The goal was to identify concentration levels that resulted in a mortality rate between 25% and 75%. The determined effective concentrations for eucalyptus essential oil were 1,000 ppm and 20,000 ppm, while for thyme essential oil, they were 4,000 ppm and 24,000 ppm. Using the logarithmic distance formula, intermediate concentrations between these values were calculated for further testing [13, 14].

Biometric tests

The biometric experiments were conducted using a completely randomized design in two phases. The first phase tested eucalyptus essential oil at six different concentrations: 1,000, 3,000, 8,000, 12,000, 16,000, and 20,000 ppm. In the second phase, thyme essential oil was evaluated at 4,000, 8,000, 10,000, 15,000, 20,000, and 24,000 ppm. Each concentration was tested in four replicates.

The immersion model (leaf dipping technique) was used for the experiment. Potato leaves, approximately 6 cm in diameter, were submerged in each essential oil solution for 30 seconds. After being removed, the leaves were left to dry for 30 minutes. Once dried, each leaf was placed individually in an 8 cm Petri dish, where a 1 cm layer of 1% agar was added to the bottom to maintain moisture.

Next, 15 aphid nymphs, precisely 48 hours old (2 days), were carefully transferred onto the treated leaves using a fine brush. The Petri dishes were then covered with fine mesh netting and kept under controlled environmental conditions: 25 ± 1 °C, relative humidity of 65 ± 5%, and a 16:8 hour light-dark photoperiod. Mortality rates were assessed 24 hours after treatment, with the number of dead aphids recorded for each Petri dish [15-17].

Results and Discussion

Effect of eucalyptus essential oil toxicity on potato aphid mortality

The statistical analysis of different conducted to assess the impact of different eucalyptus essential oil concentrations on potato aphid mortality (using the water distillation model) yielded an F statistic of 681.722, with a significance level of $P < 0.01$ (0.000). These results confirm a statistically significant difference among the tested concentrations.

Furthermore, the findings indicate a direct correlation between increasing eucalyptus essential oil concentration and aphid mortality. As the concentration rises, the percentage of aphid fatalities also increases. The highest recorded mortality rate (88.71%) was observed at a concentration of 20,000 ppm, while the lowest mortality rate (20.37%) was recorded at 1,000 ppm **(Figure 2)**.

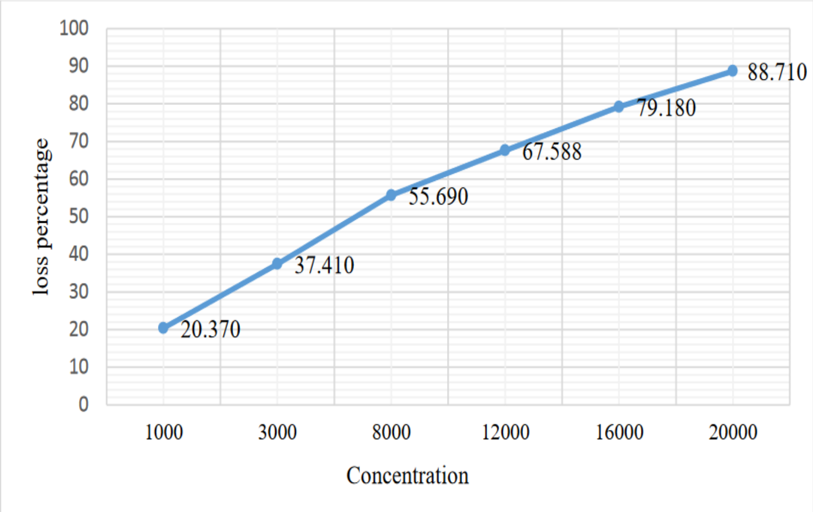

Figure 2. Loss percentage is caused by various concentrations of eucalyptus essential oil.

The statistical analysis of variance evaluating the impact of thyme essential oil at varying concentrations on potato aphid mortality (using the water distillation model) resulted in an F statistic of 571.008, with a P-value < 0.01 (0.000). These findings indicate a statistically significant difference among the tested concentrations.

The results demonstrate a clear trend: as the thyme essential oil concentration increases, aphid mortality also rises. The highest observed mortality rate (79.54%) occurred at 24,000 ppm, whereas the lowest recorded mortality rate (17.43%) was observed at 4,000 ppm.

Comparison of eucalyptus and thyme essential oil mortality rates in the water distillation method

To further evaluate the effectiveness of eucalyptus and thyme essential oils, a comparative analysis of their mean mortality rates was conducted using analysis of variance (ANOVA) and Tukey's post hoc test. The variance analysis produced an F statistic of 0.858, with a P-value of 0.466, which exceeds the 0.01 significance threshold. According to Tukey's paired comparison test, identical Latin letters indicate that no statistically significant difference exists between the mean mortality rates of the two essential oils at the 1% significance level **(Table 1)**.

Table 1. Mean mortality percentage comparison of thyme and eucalyptus essential oils

Essential Oil	Eucalyptus	Thyme
Mean mortality percentage ± SE	58.15 ± 4.92	49.13 ± 4.38
Statistical comparison of means	A	A

Probit analysis of mortality rate

The probit analysis conducted in this study determined the concentrations of eucalyptus and thyme essential oils required to achieve mortality rates of 10%, 50%, and 90% among potato aphids.

For eucalyptus essential oil, the analysis of mortality rates in the water distillation method revealed an LC50 value of 4699 ppm, with the corresponding regression equation $y = 1.428x - 5.243$. The correlation coefficient (R) was 0.985, indicating a strong linear relationship between the probit-transformed mortality and the logarithm of concentration and rate at a 99% confidence level. Additionally, the coefficient of determination ($R^2 = 0.940$) suggests that 94% of the variation in probit-transformed mortality is attributable to changes in concentration. The data confirm that increasing eucalyptus essential oil concentration leads to higher aphid mortality.

For thyme essential oil, the probit analysis determined an LC50 value of 11,944 ppm, with the regression equation $y = 2.202x - 8.978$. The correlation coefficient (R) was 0.996, demonstrating a strong linear relationship between concentration and mortality at a 99% confidence level. The coefficient of determination ($R^2 = 0.976$) indicated that 97.6% of the variation in mortality could be explained by concentration changes. The findings suggest that thyme essential oil also exhibits a dose-dependent increase in insecticidal effects.

Comparing eucalyptus essential oil with chemical insecticides

A field comparison between eucalyptus essential oil and conventional imidacloprid insecticide for potato aphid control revealed a significant difference in efficacy. The mortality rate for imidacloprid ranged from 91.4% to 98.32%, while eucalyptus essential oil resulted in a mortality rate between 46.25% and 53.16%. These results suggest that while eucalyptus essential oil is effective, increasing its concentration could enhance its pesticidal efficiency.

Given the rising costs of chemical pesticides and their associated environmental risks—including pest resistance, toxic residues in ecosystems, and harm to beneficial organisms—plant-derived essential oils offer a promising alternative. Their biodegradability, lower toxicity to non-target species, and reduced environmental persistence make them an attractive option for sustainable pest management.

Significance of plant-derived essential oils in aphid control

Aphids pose a major threat to potato crops, not only by feeding on plant sap and reducing yield but also by transmitting multiple viral diseases. Identifying plant-based compounds with insecticidal properties is crucial in mitigating this threat. The findings of this research demonstrate that the tested essential oils significantly reduce potato aphid populations under field conditions. The aphids exhibited immediate behavioral changes upon exposure to these compounds, including heightened activity and erratic movement, followed by progressive immobility and eventual death. A detailed analysis confirmed a significant correlation between increasing essential oil concentration and aphid mortality rates.

Notably, eucalyptus essential oil, with an LC50 of 4699 ppm, exhibited 2.5 times greater toxicity than thyme essential oil (LC50 of 11,944 ppm), highlighting its superior efficacy in aphid control.

Previous research supporting plant essential oils as bioinsecticides

The insecticidal properties of essential oils against aphids have been widely investigated. Abualfia and Samara [18] examined the antifeedant activity of eucalyptus, rosemary, and sage essential oils against green peach aphids and their potential to induce resistance in different potato cultivars. Their results indicated that these essential oils not only deterred aphid feeding but also reduced the transmission of viral pathogens to potato plants [18, 19].

In another study, Zhou *et al*. [20] assessed the toxicity of nine monoterpenes—key constituents of essential oils—against *Myzus persicae (*Sulzer). Their bioassays revealed that all tested monoterpenes exhibited insecticidal properties, with mortality rates increasing in a dose-dependent manner. This suggests that essential oils and their active components can serve as plant-based insecticides for aphid management [20-23].

Furthermore, studies have shown that essential oils extracted from black seed, sedum, and chamomile plants effectively target cabbage wax aphids and bean aphids (Pymetrozine Dinotefuran), further supporting their role as potent botanical insecticides [24, 25].

This research highlights the potential of eucalyptus and thyme essential oils as natural alternatives to chemical insecticides in controlling potato aphid populations. The findings reinforce the efficacy of plant-derived compounds in reducing pest pressure while minimizing environmental and health risks. Future studies should explore higher concentrations, formulation improvements, and integrated pest management strategies to enhance the practical application of these essential oils in sustainable agriculture.

Conclusion

The growing concern over human health, environmental safety, and the demand for healthy products has driven the need for alternative pest control methods that reduce reliance on chemical pesticides. Plant-derived essential oils emerge as a viable substitute, thanks to their repellent, insecticidal, and anti-nutritional characteristics. Furthermore, essential oils are naturally occurring substances, environmentally friendly, and free from harmful side effects.

This study demonstrated that both thyme essential oils and eucalyptus possess notable insecticidal activity against potato aphids. A clear and significant correlation was observed between the concentration of essential oils and the mortality rate of aphids: higher concentrations of the oils led to increased aphid mortality. Additionally, when comparing the two oils, eucalyptus essential oil exhibited higher toxicity, as evidenced by its lower LC50 value compared to thyme essential oil.

These findings underscore the considerable potential of plant essential oils in controlling aphid populations. With further research, especially through combining different essential oils, these natural compounds could serve as a promising alternative to conventional chemical pesticides, offering a safer and more sustainable option for pest management.

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