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Investigating the Insecticidal Effects of Two Essential Oils of Thyme and Eucalyptus on *Macrosiphom Euphorbiae*

Sabrine Attia[1-3*], Georges Lognay[2], Stéphanie Heuskin[2], Thierry Hance[1]

[1]Earth and Life Institute, Biodiversity Research Center, University catholic of Louvain, B- 1348 Louvain-la-Neuve, Belgium.
[2]Chemical Analysis Laboratory, Gembloux Agro-Bio Tech, University of Liege, B-5030 Gembloux, Belgium.
[3]Research Institute of Insect, Biology. CNRS UMR 7261, University of Tours, 37200, Tours, France.

ABSTRACT

Due to low toxicity, fast decomposition, and compatibility with the environment, plant essential oils are used as a suitable alternative strategy for pest control. The purpose of this study was to investigate the insecticidal effects of two plant essential oils, Eucalyptus and Zataria, on *Macrosiphom euphorbiae*. In this study, plant essential oils were extracted using a Clonger device by water distillation method. Bioassay tests were performed on adult insects at different concentrations for eucalyptus and thyme essential oils. According to the results of probit data analysis, LC50 and LC90 values for eucalyptus essential oil were 4699 and 37106 ppm, respectively, and for thyme essential oil, these values were 11944 and 45620, respectively. The results of this research indicate that eucalyptus essential oil has higher toxicity than thyme essential oil and its lethality is also higher. In this study, the highest lethality (percentage of casualties) was obtained for eucalyptus essential oil at a concentration of 20,000 ppm, and for thyme essential oil at a concentration of 24,000 ppm. Considering that the LD50 of eucalyptus essential oil and thyme essential oil is lower than the dosages of chemical insecticides and due to the emergence of the phenomenon of resistance to the effect of chemical insecticides, it seems that the essential oils of plants such as eucalyptus and thyme are suitable alternatives to chemical insecticides in the future.

Keywords: Essential oils, Insecticidal effects, Thyme, Eucalyptus.

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Corresponding author: Sabrine Attia
E-mail ✉ sabine_bio5@yahoo.fr
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INTRODUCTION

The natural and human hazards caused by the use of chemical insecticides to reduce pest damage for the growing human population have increased significantly. The widespread use of these compounds has caused resistance in pest insects, contamination of human food, poisoning of mammals, impact on non-target organisms, and environmental pollution. Due to the recognition of the dangers caused by the use of chemical insecticides on the environment and humans, the trend towards alternatives with similar efficiency for controlling agricultural pests has increased [1, 2], one of these alternatives is natural and does not have Plant compounds are dangerous for the environment and humans. On the other hand, one of the most important agricultural products around the world is potato, and due to the presence of various energy-generating substances, sugar, protein, and vitamins, it is considered one of the strategic products and the same row as wheat, barley, and rice in the world. To the extent that the World Food Organization called 2008 the year of the potato. Almost one-third of this product is produced in developing countries, mainly in Asian countries [3, 4].

Important pests damage the potato crop worldwide. Aphids are one of the most important

pests. By feeding on plant sap, aphids can cause its weakness, reduce the quantity and quality of agricultural products, and damage it [5]. In addit ion, they can transmit several important potato viruses. High populations of aphids have a direct effect on host performance. Usually, the first issue about aphids is their role as carriers of viruses, at least 10 important viral diseases of potatoes are transmitted by aphids, which cause significant damage, reduced yield, and increased costs of apple production. It becomes terrestrial [6].

Currently, chemical control is the most common method of potato aphid control. Indiscriminate use of chemical insecticides while creating pest-resistant populations in different parts of the world has had numerous side effects on the environment and non-target organisms. Due to the resistance of potato aphids to common chemical pesticides, in recent years there has been a tendency to use insecticidal compounds of plant origin to control potato aphids. Therefore, it seems that plant compounds can be used as an alternative or supplement to chemical insecticides to protect agricultural products in the future. These compounds do not have negative effects on nature and are much safer than chemical insecticides, they decompose quickly, they do not remain in the soil and water, and they can also not have a strong negative effect on the population of non-target insects [7-9].

The purpose of this research is to investigate the insecticidal effects of the essential oils of two important medicinal plants and their effects on roasted potatoes to control pests and reduce environmental hazards caused by the use of chemical insecticides.

MATERIALS AND METHODS

To carry out this research, the leaves of the tested plants were collected during the flowering of eucalyptus and thyme. The leaves were dried in the shade and at room temperature with proper ventilation before essential oil extraction. Then, the collected plants were extracted with essential oil by the Clevenger machine by water distillation method **(Figure 1)**.

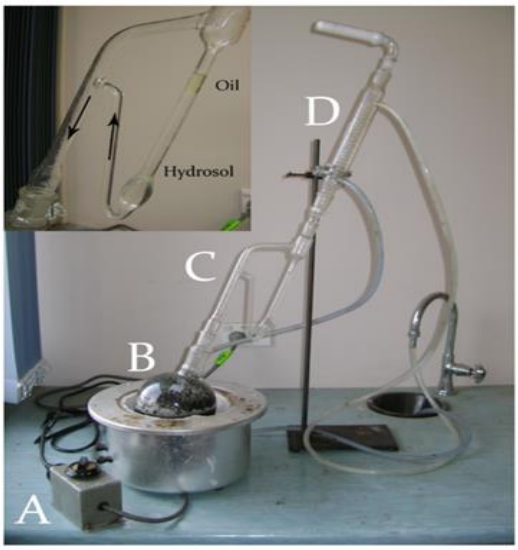

Figure 1. Water distiller (Clevenger); a) Power regulator, b) Heating mantle with round bottom flask containing water and aromatic leaves, c) Clevenger-type apparatus which returns the hydrosol to the still and maintains the essential oil phase, but only for essential oils that are less dense than water and therefore float, d) The condenser.

Collection and cultivation of potato aphid colony

The collection of aphids started with the appearance of the pest on the potato plants of the tested field. The collected aphids were placed on the leaves cut from the plants in the field to maintain the aphid population for the next experiments. These leaflets were placed inside Petri dishes containing soaked cotton and filter paper, and then the Petri dishes were transferred to the growth room. The existing leaflets were replaced with new leaflets every two days. Before starting the experiment, these aphids were transferred to the potato plants grown in the greenhouse.

Cultivation of the host plant

For this purpose, 10 plastic pans with a circular circumference of 50 cm in diameter and 15 cm in height were filled with a mixture of 2/3 agricultural soil and 1/3 sand, and one tuber of Sprite cultivar was planted in the circumference of each pan. These pots were grown in greenhouse conditions and irrigated once every four days, and urea fertilizer was used in their soil once during the experimental period. When the plants reached the stage of five to six leaves, the leaves were removed from the plants. In this way, the leaflets from the second and third compound leaves were cut from the top of the

plants and used in the experiment. To prevent aphids from escaping, these pans were enclosed by transparent plastic arches, which were connected to the upper part of the silk net fabric.

Homogenization of aphids

To perform biometric experiments, it is necessary to have adult aphids of the same age, for this purpose, the leaf disc method was used [10]. In this way, several mobile adult aphids were removed from infected potato plants with a fine brush and transferred to healthy potato leaf discs that were placed in Petri dishes with a diameter of eight centimeters. Inside the Petri dishes to maintain humidity, some cotton moistened with distilled water was placed, and then a filter paper with a diameter of 9 cm was placed on the cotton so that it was completely placed in the environment inside the Petri dish. The filter paper was used to prevent the legs of aphids from getting stuck while moving inside the Petri dishes, and a leaf cut from potato plants was placed on the filter paper so that the back surface of the leaf was facing up. About 10-20 whole aphid insects were placed on each leaf disk. A hole with a diameter of two centimeters was made in the lid of the Petri dishes and a net cloth was installed on it. The aphids were given 24 hours to produce nymphs. After the specified period, the adult aphids were removed from the leaf disks, and the nymphs of the same age on the leaf disks were allowed to grow in the same conditions. After 7-8 days, when the nymphs turned into adult aphids, they were used for biometric experiments. The aging process was carried out in an incubator with a temperature of 25 ± 1 degrees Celsius and a relative humidity of 65 ± 5 % with a photoperiod of 16 hours of light and 8 hours of darkness [11, 12].

To obtain suitable concentrations of plant essential oils that show the death rate of potato aphids, several preliminary experiments were done. The purpose of these tests was to obtain concentrations of the mentioned essential oils that can cause losses in the range of 25 to 75%, which for eucalyptus essential oil is 1000, 20000 ppm and for thyme essential oil 4000, 24000 ppm respectively. M was obtained. Then, using the logarithmic distance formula, the concentrations between them were determined [13, 14].

Biometric tests

After determining the main concentrations of the biometric tests in a completely random design and in two stages, first with six concentrations of 1000, 3000, 8000, 12000, 16000, and 20000 ppm for eucalyptus essential oil and then with concentrations of 4000, 8000, 10000, 15000, and 20000 24000 ppm for thyme essential oil was done in four replicates. The immersion method (immersion of leaves) was used to experiment. For this purpose, the leaves of the host plant (potato) with a diameter of about 6 cm were immersed in the desired concentrations of each of the essential oils for 30 seconds and after half an hour, the surface of the leaves was dried and then each The leaves were individually transferred into Petri dishes with a diameter of 8 cm, on the bottom of which 1% agar was poured to a height of 1 cm to keep the leaves moist. Then, 15 aphid nymphs at 48 hours of age (2 days) were transferred to the treated leaves in a petri dish with a brush. Petri dishes were also covered by thin netting. Petri dishes were kept in the incubator with constant conditions of 25 ± 1 degrees Celsius, relative humidity of 65 ± 5%, and photoperiod of 16:8 hours (light: dark). After 24 hours, the number of casualties in each container was directly counted and recorded [15, 16].

RESULTS AND DISCUSSION

Investigating the effect of Eucalyptus essential oil toxicity on potato aphid mortality

Based on the analysis of variance conducted to compare the average percentage of losses caused by different concentrations of eucalyptus essential oil on potato aphids in the water distillation method, the F statistic is equal to 681.722 and the corresponding significance value is less than 0.01 (0.000) Was obtained. Also, the examination of the results of the effect of different concentrations of eucalyptus essential oil on the mortality of aphids shows that with the increase in the concentration of eucalyptus essential oil, the percentage of casualties increases, so that the highest percentage of casualties (88.71%) corresponds to the concentration of 20,000 ppm and the lowest loss percentage (20.37%) corresponds to the concentration of 1000 ppm.

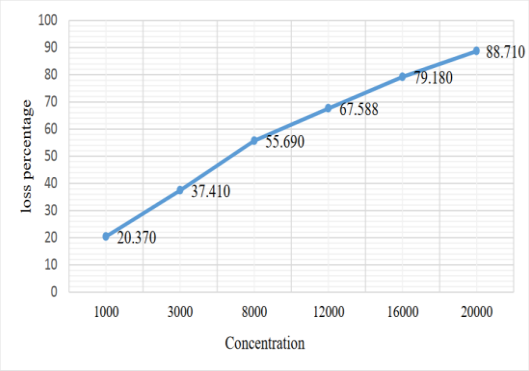

Figure 2. Comparing of loss percentage caused by various concentrations of eucalyptus essential oil.

Investigating the effect of thyme essential oil toxicity on potato aphid mortality

Based on the analysis of variance conducted to compare the average percentage of losses caused by different concentrations of thyme essential oil on potato aphids in the water distillation method, the F statistic is equal to 571.008 and the corresponding significance value is less than 0.01 (0.000). Examining the results of the effect of different concentrations of thyme essential oil on the percentage of losses shows that with the increase in the concentration of thyme essential oil, the percentage of losses increases so that the highest percentage of losses (79.54%) corresponds to the concentration of 24,000 ppm and the lowest (17.43%) corresponds to the concentration of 4000 ppm.

Comparing the average losses of eucalyptus and thyme essential oils in the distillation method with water

In the following, the average losses of two essential oils eucalyptus and thyme were compared with water in the distillation method using analysis of variance and Tukey's post hoc test. According to the results of the analysis of variance, the F statistic is equal to 0.858 and the corresponding significance value is greater than 0.01 (0.466). In Tukey's paired comparison test, similar Latin letters indicate the absence of a significant difference between the means at a significance level of 1% **(Table 1)**.

Table 1. Comparing the losses percentage average of thyme and eucalyptus essential oils.

	Eucalyptus	**Thyme**
Average percentage of losses +SE	58.15 ± 4.92	49.13 ± 4.38
Comparison between means	A	A

Probit analysis of loss percentage

In the experiment conducted using probit analysis, a concentration of eucalyptus and thyme essential oils that caused 10%, 50%, and 90% losses was also calculated.

For eucalyptus essential oil, according to the results obtained from the probit analysis of the percentage of losses in the water distillation method, the LC50 value was obtained as 4699 ppm, and the equation of the line is y=1.428x-5.243. The obtained correlation coefficient (R) is 0.985, which shows a linear relationship between the logarithm of the concentration and the probit of the loss percentage with a probability of 99%. The coefficient of determination (R^2) equal to 0.940 has been obtained, which shows with a probability of 99% that 94% of the average probit changes are related to changes in the concentration logarithm. Based on the results, with the increase in the concentration of eucalyptus essential oil, the amount of losses increases.

For thyme essential oil, according to the results obtained from the probit analysis of the loss percentage in the water distillation method, the LC50 value was obtained as 11944 ppm, and the equation of the line is y = 2.202x-8.978. The obtained correlation coefficient (R) is 0.996, which shows a linear relationship between the logarithm of the concentration and the probit of the loss percentage with a probability of 99%. The coefficient of determination (R^2) is equal to 0.976, which shows with a probability of 99% that 97.6% of the average probit changes in the percentage of losses are related to changes in the logarithm of concentration. Based on the results, with the increase in the concentration of thyme essential oil, the loss shows a uniform increase.

Comparing the effectiveness of the optimal eucalyptus essential oil with common chemical insecticides in controlling potato aphids in field conditions showed that the efficiency percentage of imidacloprid poison varied between 91.4 and 98.32%, and this rate for eucalyptus essential oil was between 46.25 and 53.16% observed that better results can be achieved by increasing the concentration of the essential oil used. On the other hand, considering the high costs of using chemical pesticides and the multifold increase in the price of pesticides in recent years, as well as the residual risks of chemical pesticides for the environment, humans, and other mammals and

the occurrence of resistance in the target pest, it seems that essential oils Due to the many benefits that plants have over chemical compounds, their potential can be used to control pests and diseases of other plants.

Considering that aphids are one of the most important pests, especially in potato fields, and at least several important potato viral diseases are transmitted by aphids, therefore, it is very important to find plants that have toxic properties against this pest. Based on the results of this research, it was found that the tested plant essential oils have a significant killing effect on potato aphid adults in field conditions, and this effect is good from the very first moments with the insect's vibrancy and rapid movements as soon as it is placed. It can be observed that when exposed to specific plant essential oils, with time and depending on different concentrations, first the insect's movements are few and gradually cease to move, and then all the vital signs stop and the insect dies. In a more detailed investigation, it was found that the percentage of deaths for both essential oils in different concentrations differed significantly with the increase of the concentration, the percentage of deaths also increased significantly [6, 7, 17]. In addition, the results show that eucalyptus essential oil with LC50 equal to 4699 ppm has higher toxicity than thyme essential oil with LC50 equal to 11944 ppm and its lethality is 2.5 times higher compared to thyme essential oil.

The effect of plant essential oils in pest control, especially aphids, has been investigated by various researchers. In research, Aboalfayah and Samara [18] investigated the anti-nutritional activities of different essential oils of eucalyptus, rosemary, and sage against green peach aphids and studied their potential role in creating plant resistance in different potato cultivars. The results showed that the mentioned essential oils have anti-nutritional and insecticidal properties against peach green aphids and prevent the transmission of viral diseases to potato plants [18, 19].

In the study by Zhu *et al*. [20], they evaluated the toxicity of 9 monoterpenes (the main components of essential oils) on *Myzus persicae* (Sulzer). Bioassay results showed that the 9 tested monoterpenes had different degrees of insecticidal activity against aphids and death and the mortality caused by monoterpenes generally increased with increasing dosage. As a result, essential oils and their components can be used as herbal insecticides against various types of aphids [20-23]. In addition, the results showed that the essential oils extracted from the leaves of the black seed, sedum, and chamomile plants are effective against cabbage wax aphids and bean aphids (Pymetrozine Dinotefuran) and as plant insecticides have a high potential in controlling various types of have aphids [24, 25].

CONCLUSION

The desire to preserve human health and the environment and produce healthy products has increased efforts to find new methods to reduce the use of chemical pesticides in pest control. Plant essential oils can be a suitable alternative to chemical poisons due to their insecticidal, repellent, and anti-nutritional properties. In addition, plant essential oils are compounds of natural origin, safe for the environment, and have no side effects. Based on the results of this research, it was found that eucalyptus and thyme essential oils have a good insecticidal effect on potato aphids, and there was a positive and significant relationship between the death rate (percentage of casualties) and the concentration of each essential oil, that is as the concentration of essential oil increased, the death rate also increased. Further, by comparing the mortality rate of two essential oils and their LC50, it was found that eucalyptus essential oil has a higher toxicity than thyme essential oil. Also, the results of this research showed that the plant essential oils used have a significant capacity to control aphids, and by conducting additional studies and combining the extracted essential oils, they can be used as an alternative to common chemical poisons in controlling aphids and other pests benefited and created a new horizon for the production of effective and low-risk poisons.

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