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Evaluation of the Effect of Some Plant Extracts Using Citronella Oil for Controlling Ants (*Pheidole megacephala*) Infesting Honeybee Apiaries

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ABSTRACT

Many apiaries in most areas of the world are being destroyed due to repeated ant attacks, in addition to the recent decline in European honeybee productivity resulting from pollution and numerous environmental changes. This impact has been observed both economically and in plant production, as bees are the most important insect species for pollinating crops worldwide, and there is no effective and environmentally safe alternative. Therefore, this study proposed the use of citronella essential oils and tested them as a repellent or retardant. Three concentrations of oil—25,000, 50,000, and 100,000 ppm—were used to test the effectiveness of the essential oil as a repellent, while chicken gizzards were used as an ant-attractant. Furthermore, the extract was tested near the beehive's exit openings to prevent ants from entering the hive and attacking the larvae. The results showed that both concentrations—50,000 and 100,000 ppm—were very strong repellents, with no significant differences observed in statistical analysis. However, differences were found between these concentrations and other treatments, including the control. Therefore, we recommend using the 50,000ppm concentration in honeycomb areas because it produces excellent results in repelling ants, as they were observed avoiding approaching 50 cm from the beehive openings. Furthermore, this concentration did not affect bee activity during field monitoring.

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1- Introduction

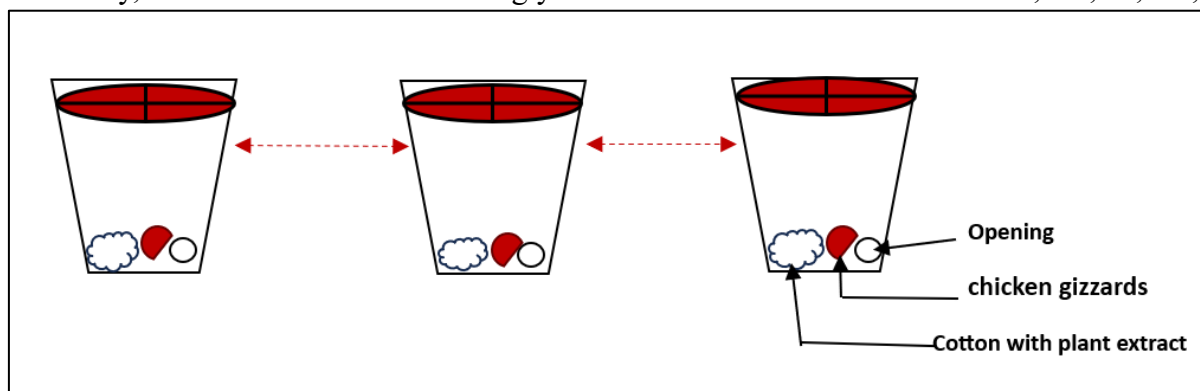
Apis mellifera Linnaeus, 1758, a honey bee from Europe (Hymenoptera, Apidae). It is one of the main species that pollinates the majority of plants globally. (1)(2). The European honey bee is considered one of the most common species for pollinating crops in most parts of the world (3). It has been observed that this species of bee is considered the most effective visitor to crops worldwide, with its flower visits reaching approximately 13% compared to other wild species (4). It is widely used for crop pollination worldwide, in addition to honey production (5). This species of bee contributed approximately \$11.68 billion to US agriculture in 2009 (6). According to the findings discussed, this honeybee species is very important for agriculture, the economy, and the environment. The majority of nations have seen a dramatic drop in honeybee populations over the past ten years. Although this decline is not unique, it has had an

impact on the amount and quality of honey produced in the majority of countries. (7)(8). According to the FAO, there was a noticeable drop in honeybee populations between 1961 and 2007. The number of colonies fell by 49.5% in North America, 42.6% in Asia, 13% in Africa, and 26.5% in Europe. Numerous biological variables, including direct reasons like infection with different diseases and pests, such as mites, or bacterial, viral, or fungal infections, cause honey bee populations to drop and, consequently, their productivity to decline (7), or non-living factors such as pollution or climate change, in addition to pesticides, etc. There are also indirect causes, such as exposure of honey bees to predators, including ants, *Pheidole megacephala*. Ants are among the most widespread species on Earth, comprising approximately 50% of the world's eukaryotic organisms (9). Large numbers of ants have been observed worldwide except for the poles, and more than 1,300 species have been identified and studied worldwide (10). The widespread distribution of ants is attributed to their social organization, their ability to adapt and renew their livelihoods to environmental conditions, their ability to exploit all available resources, their superior defense capabilities, and their ability to carry objects twice their weight (11)(12). *Pheidole megacephala*, a species closely associated with humans, travels the world, hiding in plant products and packaged materials such as construction supplies, logging materials, and military equipment. More than 150 species of ants have been introduced and spread throughout the world by human activity, where they have spread rapidly and become the most invasive and destructive species of all time (13). This study focused on the ant *Pheidole megacephala*, an ant native to tropical regions, which has become one of the most successful invasive ants due to its high predatory capabilities and successful recruitment methods (14). The activity of this type of ant extends in hot, humid climates, that is, from the end of August to October, where the highest activity of the workers of this type of ant is in the early morning and gradually decreases in the evening (15). The ants invade and destroy beehives, causing great damage by feeding on their larvae and stealing the honey produced (16). Many methods have been followed to combat this type of ant, including a study conducted in 1984 in pineapple fields, where the ants were controlled using a pesticide containing an active ingredient. Effective control of the workers of this type was observed, which reduced the economic damage to this crop for long periods (17). Many laboratory studies were also conducted to test a number of pesticides to control the ants. During this study, baits were prepared using corn and soybean oil with different proportions of pesticides to determine the direct toxicity to the ants and the ability of the bait to attract ants (18). A study was also conducted to control *Pheidole megacephala* that were invading laboratories and insect colonies, using bait containing Methoprene, a genetic hormone, to disrupt the life cycle of this type of ant (19). The big-headed ant and the tuberculosis bee have an ancient history of co-evolution that has led to many developments and adaptations between predator and prey. If the honey bee developed many defenses to confront enemies (20), while the ants also developed their capabilities for invasion and predation with many behavioral traits and adaptations, as they have a large head in addition to having strong jaws and the ability to sting poisonously (9). Active organic compounds produced by some plants can be used as killers, repellents, or inhibitors of some types of insects (21)(22). So, the citronella herb was chosen, which belongs to the family (Poaceae / Gramineae). This herb produces aromatic oils by steam distillation. This oil is widely used in all areas of life; it is considered a source of active organic compounds such as citronellol, citronellal, and geraniol, which are used in many areas such as the soap and cosmetics industry. This herb is grown in tropical and subtropical regions in Asia, America, Africa, Malaysia, and West India (23). Approximately 300-350 tons of this herb oil have been produced in India over the last six years (24). Due to the toxicity of this oil to a large group of insects, it has been widely used in combating harmful insect pests (25). Other insect species that harm honey beehives economically, including the ant *Pheidole megacephala*, will be tested in this study, as well as a program that lessens their negative effects on beehive output.

2- Materials and Methods

2-1 Preparation of the Plant Extract

Citronella leaves were used to make the plant extract, also known as citronella essential oil. A steam distillation apparatus was used to extract the essential oil (26). After evaporating the water in a different vessel, the steam was directed over the plant leaves, which were then put in a different vessel and allowed to be exposed to the steam. After the steam went through a condenser, the condensed liquid was gathered using a funnel to separate the water and oil. Following extraction, the essential oil was gathered and kept at 4°C in glass bottles. Citronella essential oil was diluted with distilled water in a 50:50 ratio to minimise viscosity, and then it was mixed with a glycerol stabiliser at concentrations of 25,000, 50,000, and 100,000



ppm to create the plant extract concentrations. Glycerol functions as an excellent stabiliser for the essential oil utilised in the experiment and has a slow evaporation boiling point of 290°C.

2-2 Test of Extract Efficacy

Between October 22 and 24, 2024, a test was conducted in an orchard in Al-Qurna District, Basra Governorate, to evaluate the efficacy of citronella essential oil in deterring and repelling ants (*Pheidole megacephala*). The humidity ranged from 46% to 55%, with the highest temperature recorded at 41.0°C and the lowest at 26.3°C. Chicken gizzards were selected as the ant-attracting bait for this experiment. To make it easier for ants to enter, the baits were put in plastic containers with holes. The bait was positioned adjacent to cotton that had been soaked in the plant extract at three predetermined concentrations for the study: 25,000, 50,000, and 100,000 parts per million. To compare the efficacy of the essential oil, a control treatment was carried out using a

Figure 1 shows the design of an experiment to determine how well a plant extract works on ants.

glycerol emulsion diluted with water in the same ratio as the essential oil. For three days, from 8:00 a.m. to 2:00 p.m. (the ant activity period), three repetitions of each concentration and control test were carried out. Two meters separated each of the plastic containers that were positioned immediately on the ground at equal intervals (Figure 1). The number of ants entering the containers was counted to record the data, accounting for the bait's renewal and the cotton's moistening by adding more plant extract every two hours. To be sure the results were accurate, the experiment was conducted three times.

2-3 Citronella Essential Oil's Impact on Employee Honeybees

We performed this experiment in a private apiary in Al-Qurna District (Figure 2), Basra Governorate, Iraq, using the same concentrations used in the previous experiment to examine the essential oil's ability to repel *Pheidole megacephala* that enter honeybees as well as its effects on honeybees. Each concentration was tested in three replicates, along with a control treatment. After moistening cotton with the extract (in the control treatment, glycerin diluted with water was used in its place), the cotton was put in Petri dishes and fastened to the sidewalls of the beehive close to the hive opening. Ants were repelled by the oil in all duplicates and at all concentrations (25,000, 50,000, and 100,000 ppm). The hives were positioned one meter above the ground, and the distance between them was set at two meters for all replicates and treatments (Figure 3). Between 7:00 a.m. and 3:00 p.m., which is thought to be the peak activity period for both *Pheidole megacephala* and worker bees, the number of bee larvae destroyed by *Pheidole megacephala* was counted (27). In every copy, the cotton was kept moist every two hours. The average number of larvae destroyed by the ants was determined after the experiment was conducted three days in a row for each replicate.



Figure 2: Honey bee hives in the Al-Qurna district in Basra Governorate that were tested

2cm

1 cm

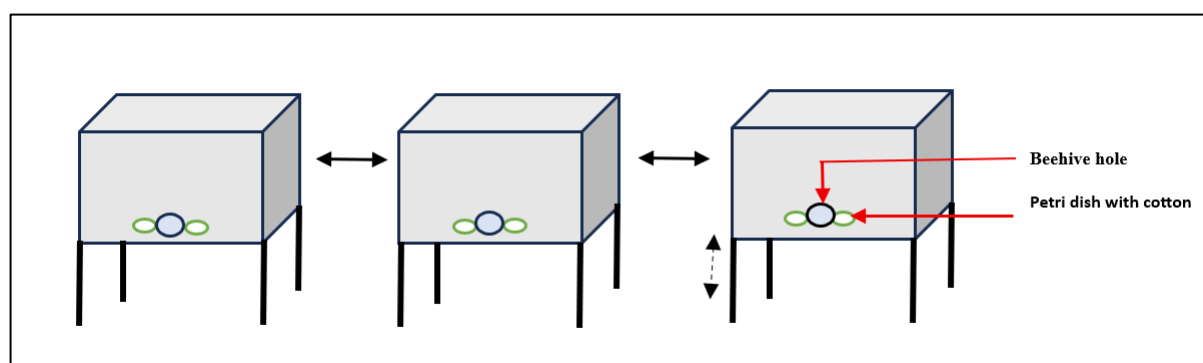


Figure 3: illustrates how well plant extract works to prevent ants from destroying honeybee larvae in beehives.

2-4 Analysis of statistics

The statistical software (SPSS V.25) was used to analyse the results. In order to determine the rate of attraction for the experimental count, as well as the frequencies and murder rate with varying treatment concentrations, probability regression analysis (ANOVA) was utilised to compare continuous variables and evaluate significant differences between means ($p < 0.05$). Regression curves were then created in order to extract the correlation coefficient (R^2).

3- Results and Discussion

The study's findings (Table 1, Figure 4) demonstrated that, in addition to the control treatment, fruit ants were drawn to chicken gizzard bait that contained citronella plant extract at concentrations of 25,000, 50,000, and 100,000 ppm. For each treatment, the average number of fruit ants generated by the bait was 5.89, 1.44, and 0.11 ants each research period. Throughout the three-day trial period, the control treatment had an average of 37.78 ants.

Table 1. Number of ants attracted to chicken gizzard bait

Repetitions - Conc.		R1	R2	R3
Control	Day 1	40	42	33
	Day 2	37	29	38
	Day 3	41	35	45
25000000 ppm	Day 1	8	5	6
	Day 2	5	4	6
	Day 3	8	5	6
50000000 ppm	Day 1	2	1	1
	Day 2	1	2	2
	Day 3	1	2	1
100000000 ppm	Day 1	0	0	0
	Day 2	1	0	0
	Day 3	0	0	0

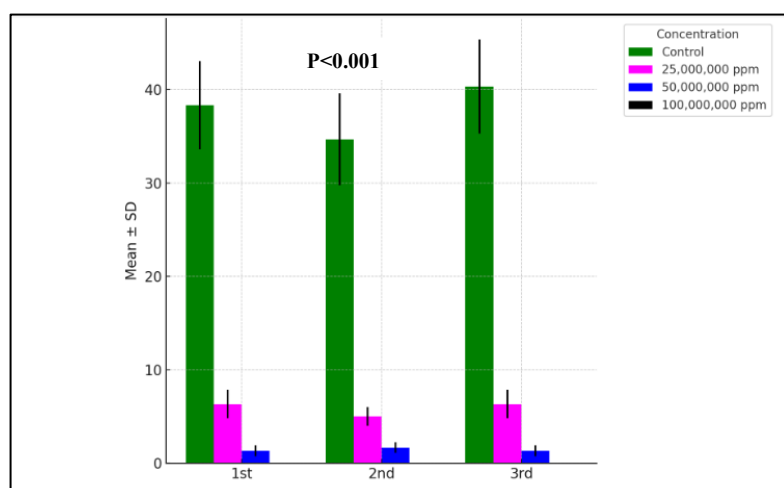


Figure 4: Ant frequency on chicken gizzards following a three-day treatment with citronella essential oil. P: two-way ANOVA test probability, significant differences $p < 0.05$. (focus * 1000)

Based on the repelling effect of the essential oil, the study's findings verify that having the extract next to the bait decreases fruit ants' interest and admission into the containers. Significant differences were found by statistical analysis between the lowest and highest concentrations (50,000 and 100,000 ppm) as well as between the control treatment and the three other treatments. The latter two concentrations, however, did not differ in any way that was statistically significant.

The number of bee larvae destroyed by the essential oil in honey beehives is displayed in Table 2 and Figure 5. For concentrations of 25,000, 50,000, and 100,000 ppm, the average number of larvae destroyed during the three days was 5, 0.33, and 0 larvae/study period. The control treatment had an average of 10 larvae per research period. By raising the concentration of the essential oil utilised, which lowers the amount of bee larvae destroyed, the repellents made from this plant are successful in keeping fruit ants away from honey beehives, according to the results of the current study. Significant differences were also found by statistical analysis between the lowest concentration and the two highest concentrations of 50,000 and 100,000 parts per million, as well as between the control treatment and the three other treatments. The two greatest concentrations did not, however, differ significantly.

Table 2. The number of bee larvae killed by ants in this study.

Repetitions -Conc.		R1	R2	R3
Control	Day 1	15	11	10
	Day 2	12	10	11
	Day 3	13	14	10
25000000 ppm	Day 1	8	7	5
	Day 2	5	7	5
	Day 3	4	5	3
50000000 ppm	Day 1	1	0	0
	Day 2	0	1	0
	Day 3	0	1	0
100000000 ppm	Day 1	0	0	0
	Day 2	0	0	0
	Day 3	0	0	0

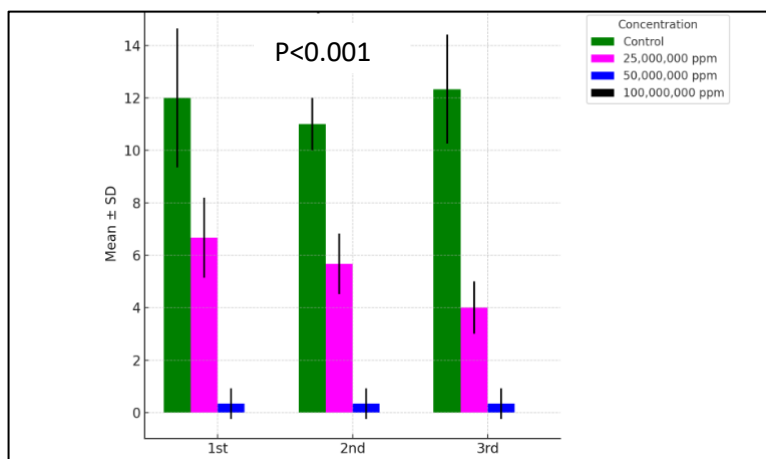


Figure 5: shows how many bee larvae were destroyed by ants following a three-day treatment with citronella essential oil. P: two-way ANOVA test probability, significant differences $p < 0.05$. (focus * 1000)

When *Pheidole megacephala* ants were observed in each treatment, it was discovered that they were extremely sensitive to the scents of the active component. At doses of 50,000 and 100,000 ppm, the average distance between them and the beehive opening was 50 cm for each replicate. Although a very small number of fruit ants reached the beehives, they lost control and were forced to move far from the apiary. This may be due to their extreme sensitivity to the essential oil used. Meanwhile, the sensing distance for the lowest concentration of 25,000 ppm was much lower, averaging 15 cm across all replicates (Figure 6). Therefore, it was observed that ants, *Pheidole megacephala*, were able to reach the apiaries, and that bee activity was not affected by the essential oil during field monitoring.

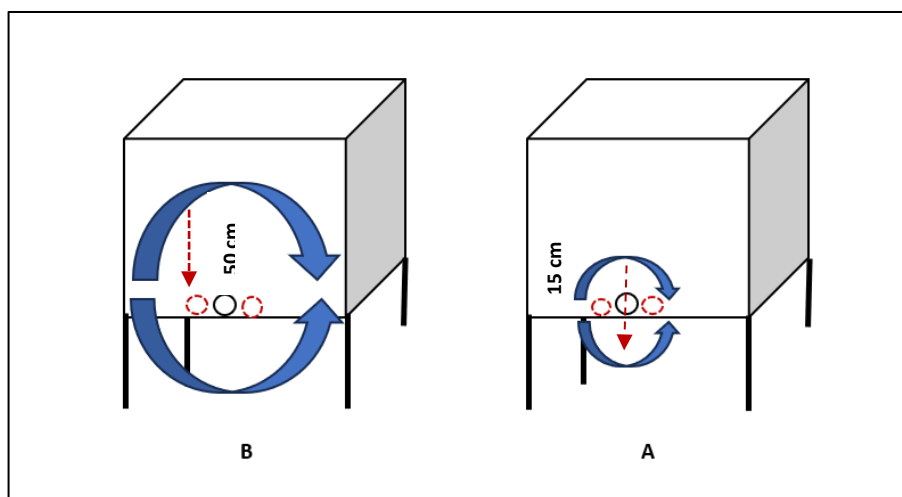


Figure 6: shows how the plant extract (Citronella oil) affects ant distances when they try to enter the beehive opening at concentrations of 50,000, 100,000 parts per million (A) and 250,000 parts per million (B).

Various studies have been conducted on the effect of citronella essential oil on many insects, but the effect of the oil on fruit ants has not yet been studied. However, its effect on a different insect pest has been studied, and for this reason, it was chosen in this study, because it has a devastating effect on the productivity of honey beehives. The study showed wonderful results that beekeepers can benefit from. Concerning previous studies that used the essential oil (28), they reported that this oil has a repellent effect on mosquitoes (*Aedes aegypti*). (29) They also found that it is effective in toxicity and repelling *Musca*

domestica (larvae and adults). Studies were also conducted on the ability of the oil to repel the *Blattella germanica* (30). In addition, a study was conducted using this oil to get rid of storage pests such as the *Sitophilus oryzae* and the *Tribolium* (31), in addition to its ability to get rid of agricultural pests as well, such as the study conducted to get rid of the *Plutella xylostella* using this oil (32). Studies showed wonderful results using the oil as a repellent and insect deterrent; Given the increasing use of essential oils in various fields, which are extracted from different plants, for example, as insecticides (33), or as preservatives or flavorings, in addition to their use in the manufacture of cosmetics and soap (34), Research is moving towards the use of natural materials rather than potentially environmentally harmful chemical ones. Lastly, because plant extracts are inexpensive, this study suggests using them more often in field investigations and applications. In addition to their positive effects, they do not negatively impact the environment.

Conclusion

The results of the investigation revealed no statistically significant variations between the control and the other treatments, as well as between the 50,000 and 100,000 ppm concentrations. In this investigation, ants exposed to the 50,000ppm dosage avoided beehive openings. Consequently, we suggest this remedy concentration for apiaries, since the oil used had little effect on local bee activity.

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