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## Insect Repellent Properties of *Melaleuca alternifolia*

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# Insect Repellent Properties of *Melaleuca alternifolia*

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**Abstract**
The aim of this study is to compare the use of plant-based insect repellents that are environment friendly with the use of insect repellents based on chemical substances which can be harmful to the environment and human health. The plant studied here is "tea tree"; its scientific name is *Melaleuca alternifolia.* Essential oil from this plant is extracted by steam distillation method. Based on the previous research, tea tree oil has antimicrobial, antifungal, anti-inflammatory, and insect repellent properties. Some experiments were done on tea tree oil to determine its insect repellent properties and the suitable concentration that can be used to make sure its repelling effect is optimum. The purpose of this determination is to avoid its harmful effect on humans because it can be toxic if it is used at high concentration. The results showed that tea tree oil repelled *Tribolium castaneum*. Furthermore, the toxicity assays also gave positive result where the tea tree oil has toxic properties against *Solenopsis invicta.* The lethal dose (LD) of tea tree oil to kill 50% of a group of *S. invicta* is 23.52 $\mu$L/mL. This $LD_{50}$ is determined by using the arithmetic method of Karber. Broadly, the results showed that *M. alternifolia* has insect repellent properties and shows toxicity against certain insects.

**Keywords:** *Melaleuca alternifolia*; Steam distillation; Repellency assay; Toxicity assay.

## 1. INTRODUCTION

A long time ago, plant-based insect repellents were used in "old style" practice, especially as protective mediums. In recent times, the wide marketing of a repellent product partially produced by a plant-based ingredient has shown increasing popularity among users. Most plants have a compound that can be used as an insect repellent.

As known, *M. alternifolia*, simply called "tea tree," is a small tree, is quite a beauty, can grow up to 5 m in height, and can have a narrow and thin bark and fine leaves that can grow up to 20 mm in length [1]. Generally, *M. alternifolia* live along narrow rivers, marshy flats, beaches and "next to" the ranges. *M. alternifolia* is Australia's native plant, and because of its vigorous growth in Australia and its properties, this plant has been widely used in Australia. We can conclude that each part of this plant can be used. In olden days, this plant was used to make medicinal ingredients, and sometimes it was used directly on the human body. Despite its usefulness, wide use of *M. alternifolia* oil did not officially begin until early this century when its antiseptic and eradicate properties were reported [1].

In recent years, an essential oil that has been extracted from the *M. alternifolia* has become increasingly used in medicine. This essential oil, which can be called tea tree oil, is a whitish yellow viscous liquid with a distinctive spicy odour and is a combination of a complex mixture of monoterpenes, 1-terpinen-4-01, cineole, and another hydrocarbon [1]. This oil that has been extracted is yielded by steam distillation process. The oil produced is comparatively low in concentration, and it is obtained at a concentration estimated at 1%-2% of the weight of wet plant material.

Several factors affect the efficiency of the distillation process, and optimizing these factors is necessary to produce a high yield of oil. Each year, the level of oil is different, and the harvesting time is an important thing that needs to be considered for the best oil-level yield. The period from November to May is the time when the level of oil yield is high.

Moreover, one of the factors that effect the final quality of oil yield is the duration of the distillation process. The oil has a number of properties which suggest its potential for use in wound treatments or as a protectant against flies. Besides, it has documented insecticidal effects by which it can be used in the treatment of strikes by larvae, a repellent effect that can help in protecting against new strikes or restrikes, and antimicrobial and anti-inflammatory effects that can help in healing wounds [2].

## 2. MATERIALS AND METHODS

### 2.1. Preparation of Essential Oil

The raw materials that have been used in this project are parts of the tea tree; its scientific name is *M. alternifolia.* The parts that were used in this experiment were the leaves from which the essential oil was extracted. The leaves were placed in the upper container of distillation apparatus with fair and tight packing. Some spaces were left to allow the steam to pass through the raw material. A low flow of water was allowed into the condenser. This allowed the oil to flow into the tube. The heating mantle

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was turned on, and steam distillation began. The heating mantle was allowed to heat the water, and the water vapour passed through the leaves. The process was carried out for a period of 30 minutes to 3 hours. The temperature was maintained at 165°C, as the boiling point of tea tree oil is 165°C. The heating mantle was turned off after the distillation process ended. The distillate was extracted further by using "liquid-liquid extraction" technique. The essential oil obtained was poured into a small bottle [3].

### 2.2. Repellency Assays

Several test samples were prepared by dissolving different volumes of the essential oil in 1 mL of acetone (5, 10, 20, and 30 $\mu$L). A Whatman filter paper was divided into two sections. Then, oil solution was applied on one half of the filter paper as uniformly as possible using a micropipette. Acetone was placed on the other half of the filter paper as a controlling agent. The essential oil–treated and acetone-treated halves were dried to completely evaporate the solvent.

After that, both treated and untreated halves were joined again by using tape and placed at the bottom of a Petri dish. Twenty insects (stored-product beetle, *T. castaneum*) were placed at the center of the filter-paper disc, and then the petri dish was covered and kept in a dark environment. For each concentration of the solution, this procedure was replicated about four times. A number of the insects on both the treated and untreated halves were recorded after four hours in dimmer light [4].

### 2.3. Toxicity Assays

Disposable Petri dishes ($100 \times 25$ mm$^2$) were prepared by covering the Petri dishes with a white cloth to prevent the escape of the ant under study and force the ant to remain on the treated paper. Several test samples were prepared by dissolving different volumes of essential oil in 1 mL of hexane (25, 30, 40, and 50 $\mu$L).

Each concentration of the essential oil was applied to the Whatman filter-paper circle (90 mm in diameter). The paper was then dried under a fume hood for 1 hour and placed in the bottom of a Petri dish. Control filter papers were treated with 1 mL of hexane. In each of five replicates, five *S. Invicta* ants were placed on top of the treated filter paper. The dish was left covered with white cloth. This method allowed assessment of mortality due to contact toxicity, as opposed to fumigation. To prevent desiccation while forcing ants to remain on the treated filter paper, water was provided in a plastic cap, cut from the top of a 0.5 mL microcentrifuge tube and placed on top of the filter paper. Mortality was assessed after every 15 min for 2 h, then finally after 24 h [5]. Plates 3.5 and 3.6 show the toxicity assay test and *S. Invicta* ant preparation, respectively.

## 3. RESULTS AND DISCUSSION

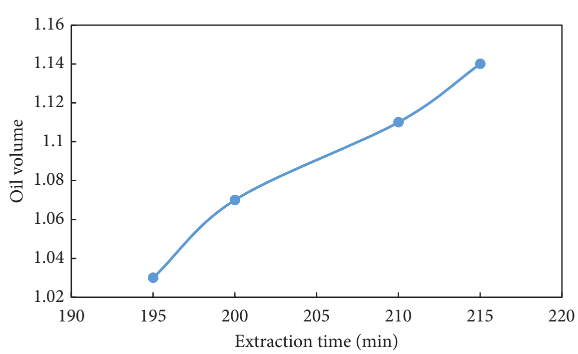
### 3.1. Extraction of Essential Oil Using Steam Distillation

The product from the steam distillation will undergo a further process, that is, liquid-liquid separation technique. When solvent (20 mL of diethyl ether) is added to the distillate (150 mL), three layers are formed—diethyl ether, essential oil, and water. From

**Table 1: The productivity of tea tree oil by steam distillation apparatus.**

| Batch | Raw material input (g) | Extraction time (min) | Oil volume (mL) | Productivity % (mL/100 g) |
|---|---|---|---|---|
| 1 | 100 | 195 | 1.03 | 1.03 |
| 2 | 100 | 200 | 1.07 | 1.07 |
| 3 | 100 | 210 | 1.11 | 1.11 |
| 4 | 100 | 215 | 1.14 | 1.14 |

**Figure 1: Tea tree oil volume yield over time.**



top to bottom are diethyl ether, essential oil, and water, respectively. This is due to the densities of each of them, which are 0.713 g/mL, 0.885-0.906 g/mL, and 1.000 g/mL, respectively. Water is discharged from the bottom of the separating funnel (250 mL), and the process of liquid-liquid separation is repeated once again to make sure all the water is removed. Although, there will be a small amount of water in the solution, most of the solution is solvent and essential oil. Anhydrous sodium hydroxide is added to remove the small amount of water, and the solvent in the solution is removed by using a hot water bath because of the low boiling point of the solvent. In steam distillation, typically the essential oil's yield is in the range of 1% to 2% of weight of wet plant material [1]. Table 1 and Figure 1 show the productivity of tea tree oil by steam distillation apparatus and the graph of tea tree oil volume yield per time, respectively.

As seen in Figure 1, the volume of essential oil yield increases as the time extraction increases, although the yield of essential oil also depends on the moisture content of the raw material (leaves). Although the volume of essential oil yield increases as the extraction time increases, it also depends on the temperature at which the mixture of raw material and steam is heated, and the temperature of the surrounding also affects the yield of essential oil. Other than that, as you can see, the extraction speed is fast at the beginning, slows down with time, and increase in the end. The average time needed to yield essential oil in this experiment is 205 min, and the average volume of essential oil yield is 1.09 mL. The total amount of tea tree oil yield is 4.35 mL.

As we know, tea tree oil has more than 100 components; the main components are monoterpenes, sesquiterpenes and their alcohols [6]. These components have been identified using gas chromatography-mass spectrometry [7]. But in the present study, all of these components have not been identified because it is not compulsory to identify them in this project. One of the objectives of this project is to extract the essential oil from *M. alternifolia* using steam distillation. Based on the result, we have achieved the objective. The present study also proves that the yield of tea tree oil is in the range of 1% to 2% of the weight of wet plant material which has been reported in the earlier journal [1].

### 3.2. Repellency Assays

This test was performed to determine the repellent activity of the tea tree oil. Chi-square test was performed to determine the repellent activity of the essential oil tested. Table 2 shows the filter paper–repellency assays using *M. alternifolia* essential oil against *T. castaneum* adults.

In this filter paper repellency assay, adults of *T. castaneum* were used. Four replications were carried out for each concentration of essential oil, and 20 *T. castaneum* adults were used per replicate. The results were expressed as mean on the untreated and treated halves in the assay. The essential oil of *M. alternifolia* tested as a repellent to *T. castaneum* adults can be indicated by Chi-square distribution analysis. Figure 2 shows the graph for repellency assay.

Line Series 1 indicates the untreated section of filter paper, and line Series 2 indicates the treated section of filter paper with tea tree oil.

From the result, it is obviously shown that *M. alternifolia* essential oil is a repellent to *T. castaneum* adults. This repels the beetles sufficiently, even at very low concentration, and the hypothesis of the ratio 1:1 was rejected. Major constituents that have

**Table 2: Filter paper repellency assays using *M. alternifolia* essential oil against *T. castaneum* adults.**

| Concentration (%) (vol:vol) | Mean of insects ± SE in untreated (%) | Mean of insects ± SE in treated (%) | $\chi^2$ value |
|---|---|---|---|
| 0.05 | 51.25 ± 0.5 | 48.75 ± 0.5 | 21.5 |
| 0.10 | 55.00 ± 0.5 | 45.00 ± 0.5 | 36 |
| 0.20 | 51.25 ± 0.5 | 48.75 ± 0.5 | 43.3 |
| 0.30 | 58.75 ± 0.5 | 41.25 ± 0.5 | 44.5 |

**Figure 2: Repellency assay.**

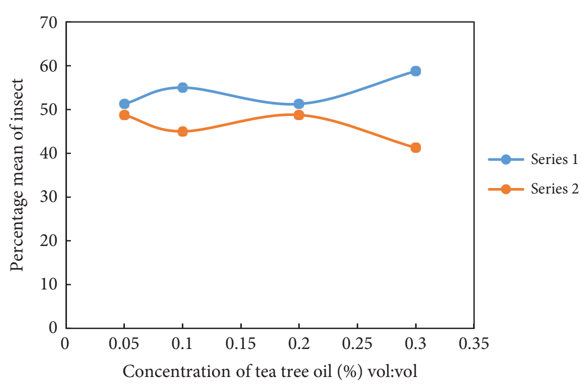



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**Table 3: Mean ± SE cumulative mortality of red imported fire ants continuously exposed to treated filter paper.**

| Time (min) Concentration ($\mu$L) | 15 | 30 | 45 | 60 | 75 | 90 | 105 | 120 | 1440 |
|---|---|---|---|---|---|---|---|---|---|
| 25 | 0.0± | 0.0± | 0.0± | 0.2± | 0.4± | 0.4± | 0.4± | 0.6± | 1.2± |
| 30 | 0.0± | 0.0± | 0.2± | 0.2± | 0.2± | 0.2± | 0.2± | 0.4± | 1.2± |
| 40 | 0.0± | 0.0± | 0.0± | 0.0± | 0.2± | 0.2± | 0.2± | 0.4± | 1.4± |
| 50 | 0.0± | 0.0± | 0.0± | 0.6± | 0.8± | 0.8± | 1.0± | 1.2± | 2.2± |
| Blank | 0.0± | 0.0± | 0.0± | 0.0± | 0.0± | 0.0± | 0.0± | 0.0± | 0.0± |

**Table 4: Mean of dead insects at different concentration of tea tree oil.**

| Group | Concentration ($\mu$l/mL) | Mean of dead insects |
|---|---|---|
| 1 | Blank | 0 |
| 2 | 25 | 1.2 |
| 3 | 30 | 1.2 |
| 4 | 40 | 1.4 |
| 5 | 50 | 2.2 |

**Table 5: Mean mortality of tea tree oil.**

| Group | Concentration difference (a) | Mean mortality (b) (second + first)/2 | Probit (a × b) |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0.6 | 0 |
| 3 | 5 | 1.2 | 6 |
| 4 | 10 | 1.3 | 13 |
| 5 | 10 | 1.8 | 18 |

repellent activity against insects are Terpinen-4-ol, y-terpinene, and $\alpha$-terpinene. For repellent and insecticidal action against *T. castaneum,* 1,8-cineole is the major constituent that is responsible for insecticidal action against *T. castaneum.* Based on Table 2, the $\chi^2$ value is the Chi-square test value, and it indicates the repellent activity of tea tree oil against *T. castaneum.* As you can see that as the concentration of tea tree oil increases, the $\chi^2$ value also increases. The result means that as the concentration of tea tree oil used increases, the repellent activity of the tea tree oil against *T. castaneum* also increases. Tea tree oil has been reported for its repellent behaviour against *T. castaneum* [7]. These earlier reports have clearly supported the result of the present study.

### 3.3. Toxicity Assays

In this test, the toxicity of the essential oil from tea tree was determined by its toxic effect against red imported fire ant, *S. invicta*. Four different concentrations were used, and each concentration was replicated five times. Table 3 shows the mean ± standard error (SE) cumulative mortality of red imported fire ants continuously exposed to filter paper treated with five different concentrations of essential oil.

Toxicity was evaluated in continuous exposure tests with treated filter paper. Based on Table 3, the filter paper treated with 50 $\mu$L concentration of essential oil is the most effective against *S. invicta* compared to the other concentrations. The mortality of *S. invicta* has been determined by calculating the percentage mean death of the insects. As you can see in Table 3, there is no insect death at the beginning. But the number of dead insects increases as the time increases. At 1440 min, for the concentrations of 25 $\mu$L and 30 $\mu$L, 24% of the insects died, and for the concentration of 40 $\mu$L, 28% of the insects died. Furthermore, at the concentration of 50 $\mu$L, the mean percentage of dead insects is about 44%. There is no dead insect in control. From the earlier study, it is seen that the mortality of tea tree oil on *S. invicta* is not very efficient [5]. The percentage mean of dead insects is about 3.3% at the concentration of 25 $\mu$l [5]. Even though the result from the earlier study is different from the present study, it is also indicated that the toxicity of tea tree oil against *S. invicta* is not very efficient. Tables 4 and 5 show the mean of dead insects at different concentrations of tea tree oil and the mean mortality of tea tree oil, respectively.

Based on Tables 4 and 5, we can find the lethal concentration ($LC_{50}$) using the arithmetic method of Karber. In this toxicity assay, $LC_{50}$ of tea tree oil against *S. invicta* is 23.52 $\mu$L/mL. From this result, we can assume that the lethal concentration of tea tree oil needed to kill 50% of a certain number of insects is 23.52 $\mu$L/mL. Lee *et al.* [8] stated that the $LC_{50}$ of tea tree oil against *S. invicta* is 22.8 $\mu$L/mL. Although the result is quite different, we can make a conclusion that the previous report has strongly supported the present study. The overall result, we can say, is that tea tree oil has a toxic effect on *S. invicta.*

## 4. CONCLUSION

Steam distillation is the best method to extract essential oil from *M. alternifolia.* The yield of essential oil depends on three factors: temperature, extraction time, and moisture content. As the extraction time increases, the yield of essential oil also increases. The lower the moisture content, the greater is the yield of essential oil. In the repellency assays, the result shows that essential oil from *M. alternifolia* is repellent and insecticidal against *T. castaneum* even at a low concentration of essential oil. The major constituent that is responsible for the repellent and insecticidal actions is 1,8-cineole. It takes times for the essential oil to affect the red flour beetle, *T. castaneum.* For the toxicity assay, the result indicates that essential oil from *M. alternifolia* is toxic against red imported fire ants, *S. invicta.* In the beginning, there is no effect on the insect, but the effect becomes clearly visible in the second hour. From these assays, it can be concluded that tea tree oil is toxic to *S. invicta.*

### Author Contributions

Each author has contributed equally in this study.

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None.

### Conflict of Interest

None.

#### References

1. Carson CF, Riley TV. Antimicrobial activity of the essential oil of *Melaleuca alternifolia*. Lett Appl Microbiol. 1993; 16(2):49-55.
2. Callander JT, James PJ. Insecticidal and repellent effects of tea tree (*Melaleuca alternifolia*) oil against *Lucilia cuprina*. Vet Parasitol. 2012; 184(2-4):271-78.
3. Stichlmair J, Fair JR. Distillation: principles and practices. Wiley-VCH, New York, USA (1998).
4. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review. Bioresour Technol. 2010; 101(1):372-78.
5. Wiltz BA. Applied ecology and control of imported fire ants and Argentine ants (Hymenoptera: Formicidae). PhD thesis (2005), University of Georgia, Athens, GA.
6. Brophy JJ, Davies NW, Southwell IA, Stiff IA, Williams LR. Gas chromatographic quality control for oil of *Melaleuca* terpinen-4-ol type (Australian tea tree). J Aric Food Chem. 1989; 37(5):1330-35.
7. Hammer KA, Carson CF, Riley TV, Nielsen JB. A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. Food Chem Toxicol. 2006; 44(5):616-25.
8. Lee BH, Choi WS, Lee SE, Park BS. Fumigant toxicity of essential oils and their constituent compounds towards the rice weevil, *Sitophilus oryzae* (L.). Crop Prot. 2001; 20(4):317-20.

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