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# Evaluation of tea tree extract formulation for the control of the cotton aphid, *Aphis gossypii* (Homoptera: Aphididae) on *Capsicum annuum* in the glasshouse

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**ABSTRACT**

Under laboratory and glasshouse conditions, the insecticidal activities of the essential oil obtained from leaves of the tea tree plant by steam distillation was evaluated against the cotton aphid, *Aphis gossypii*. The results showed that tea tree essential oil affected the aphid population causing a higher mortality of *A. gossypii*. This study showed that the 50 µL/mL concentration of tea essential oil caused 85.67% mortality after 24 hrs exposure time when used as contact treatment. However, the mortalities at 10, 20 and 30 µL/mL were 18.33, 21.67 and 70%, respectively, after 24 hrs of exposure. Conversely, the residual treatment was the most effective on the cotton aphid, with 100% mortality at the concentration of 50 µL/mL after 24 hrs of exposure to the essential oil. In contrast, the mortalities of *A. gossypii* were 15.00, 24.44 and 92.78% at the concentrations of 10, 20 and 30 µL/mL, respectively, after 24 hrs of exposure. The formulation of the tea tree essential oil was enormously potent in reducing the cotton aphid population on the sweet pepper, but was slightly phytotoxic to the potted plants. No significant differences were observed between tea tree oil formulations compared with the chemical pesticides, Karate and botanical insecticide, Levo. Based on our findings, the tea tree essential oil can be used as a botanical pesticide against *A. gossypii* after modifying it to reduce the phytotoxicity on the plants.

**Key words:** Aphids, *Aphis gossypii, Melaleuca alternifolia, Plant extract, GC-MS, Toxicity*.

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## INTRODUCTION

The cotton aphid, *Aphis gossypii* is a member of the Family Aphididae of the Order Homoptera. This species is a polyphagous pest that may be found worldwide. *Aphis gossypii* is considered a serious pest and feeds on many plant families, including vegetables, crops, and weeds. Several previous studies reported that the infestation of cotton aphids on over 500 plant species belonging to 40 plant families could cause a high economic loss on different yields (Blackman *et al.*, 2000; Ben-Issa *et al.*, 2017). *Aphis gossypii* feeds on plant fluid, which causes yellowing and curling of the plant leaves. Over 180 plant viruses have been involved in the transmission by *A. gossypii*. As a result, the cotton aphid is considered a polyphagous pest that infests more than 50 plant Families (Favret and Miller, 2012).

Plant-based biopesticides have been obtained from numerous species of plants with bioactive components that affect many insect pests belonging to 60 Families and can be used commercially in plant protection (Dev, 2017; Miresmailli *et al.*, 2006). The majority of botanicals is low to moderately toxic to the environment and break down faster than chemical pesticides (Buss and Park, 2002). High mortality of insect pests can be caused by using plant extracts in different applications such as antifeedants, repellents, and fumigants (Regnault-Roger, 1997). Some plant extracts showing insecticidal activities include those from, rosemary, oregano, neem and eucalyptus (Tunc and Şahinkaya, 1998).

*Melaleuca alternifolia* was used as a bio-insecticide because the tea tree has constituents that affect several insect pests (Choi *et al.*, 2003; Carson *et al.*, 2006). *Melaleuca alternifolia* could offer an alternative way to chemicals as a new bioactive product to control insect pests (Liao *et al.*, 2017). Several plant extracts, including those from the tea tree, have been examined against several insect pests such as thrips, mealybugs, whiteflies, and aphids. The results showed that they caused high mortality on these insect pests (Mann *et al.*, 2012).

The action of plant extracts against some insect pests is considered a new generation of natural pesticides that have less influence on the environment (El-Hosary, 2011). Chemical pesticides were used to control aphids, which when used incorrectly, can lead to environmental pollution and aphids' resistance to these agrochemicals, especially *A. gossypii* (Ben-Issa *et al.*, 2017). Cotton aphids have resistance to several pesticides and the many applications of chemicals used, such as pyrethroid groups (Chimi *et al.*, 2009). According to Isman (2017), research is needed to understand the practical application of natural pesticides under complex agro-ecological conditions. Moreover, pesticides perform when applied to various crops under different growing conditions. Their impacts on insect pests and beneficial species and their safe usage to the environment and agro-ecological advantages must be explored.

Because certain pesticides are losing their efficiency as a result of insect pest resistance, this study was conducted firstly; to determine the chemical composition of *M. alternifolia* through GC-MS analysis in order to identify its main compounds. A second aim was to investigate the effect of the tea tree essential oil on the cotton aphid *A. gossypii* by examining contact and residual toxicity as well as using the tea tree oil formulation in the glasshouse condition.

## MATERIALS AND METHODS

### Aphid's culture

The cotton aphid *A. gossypii* was reared on potted sweet capsicum, *Capsicum annuum,* plants under the glasshouse conditions using cages 40 cm x 40 cm x 40 cm covered by fine mesh cloth provided with a zip to make easy access into the cage. The temperature in the glasshouse condition for rearing colonies of aphid fluctuated between 18 and 25˚C during the day and night, and the measurement of humidity level ranged between 60 – 75% and 18 h light: 6 h dark photoperiod (Gavkare and Gupta, 2013). The HoBoware® (temperature/relative humidity data logger) was used inside the glasshouse and associated software (Onset Company, One Temp Pty. Ltd., Adelaide, Australia) to record the temperature and humidity. *Aphis gossypii* was transferred to the leaves of sweet pepper plants using a fine brush.

### Green insecticide of tea tree essential oil

The tea tree, *Melaleuca alternifolia,* pure essential oil was obtained from the leaves and terminal branches purchased from the market and extracted by steam distillation using methanol (99.9%) and hexane (97%).

Green insecticides (or botanical insecticides) were prepared from the tea tree oil. Liquid formulations were naturally prepared by mixing tea tree essential oil to make emulsifiable concentration (EC) insecticide, as follows: 200 mL of distilled water was added slowly to a 600 mL glass beaker by using a burette for 5 min, and then added emulsifiers 1% surfactants span 80, 0.5% potassium oleate and 5% vegetable oil as carrier oil was added to the 5% essential oils with continued stirring for more than 5 minutes by using a magnetic stirrer (Isaacs and Chow, 1992). Formulations were prepared by mixing tea tree essential oil with natural solvent (vegetable oil and water) with an emulsifier (span and potassium oleate) at the quantity mentioned above.

### Sweet pepper plants

All experiments were conducted on *C. annuum* plants. The capsicum seedlings were transferred and grown in plastic pots (size 1 Kg) filled with potting mix under glasshouse conditions (25±2˚C, 60±5% RH and seasonal photoperiod). All potted plants were infested by cotton aphid's *A. gossypii*, when the plant reached age eight weeks. To compare the effect of tea tree formulation as a green insecticide on cotton aphids with chemical pesticides, two agrochemicals were purchased from the local market which were Karate (Lambda-Cyhalothrin 2.5% EC, Syngenta Crop Protection, Inc) and Levo (Oxymatrine 2.4% SL, Sineria Holland BV) and three replicates of treatments were set up for each tea tree extract formulation, chemical pesticide Karate (Lambda-Cyhalothrin 2.5%), and botanical insecticide Levo (Oxymatrine 2.4%) treatments additionally to the control treatment. Data were collected after application at 2, 3 and 7 days under recommended dose.

**GC-MS analysis of tea tree essential oil**

The tea tree oil was analysed by using Shimadzu GC-MS model QP2010 series, connected with the column (SGE main category BPX5) 30 m x 0.25 mm film thickness 0.25 µm (Kinesis Australia Pty Ltd, Qld, Australia) coupled to a mass spectrum detector. The GC was connected with the AOC-5000 auto sampler (Shimadzu, Kyoto, Japan). The method of GC-MS analysis that were used: the gas carrier was helium, and the volume of sample injected 1 µL diluted with the solvent of hexane, the injector temperature was 220˚C and the pressure was 63.43 kPa; whereas, the gas flow mode was 1.07 mL/min and the linear velocity was 37.8 cm/sec. The interface temperature was 200˚C and the solvent cut time was 1.5 min. Tea tree oil was injected in two replicates and components of the analyzed oil were identified based on computer matching with commercial library and compared with the obtained results of mass spectra for each constituent with the standard in mass spectra libraries, NIST database was also used. The oil composition percentage was calculated using Microsoft Excel for the peak area (Ahmed, 2021).

**Toxicity of tea tree essential oil to *Aphis gossypii***

The tea tree oil's contact action was tested using four different concentrations. For each treatment, there were three replications. The control treatment was carried out the same way as the experimental treatment, except for the solvent. A Whatman (No. 1) filter paper was treated by spraying 1 mL of tea tree oil for each concentration using a micro-spray size 5 mL sprayer, and the filter paper was put inside a 9 cm petri dish. 20 to 30 aphids were transferred into each petri dish. After a spray of the tea tree solution on filter papers, each petri dish was covered by a piece of mesh and kept in an incubator chamber at the conditions 25±2˚C, 16 L: 8 D and 65.5% RH). After different exposure times that were 1, 3, 6, 8 and 24 hrs, mortality was recorded under a microscope.

For the residual treatment, aphids were exposed to treat Whatman (No. 1) filter papers that were individually sprayed using a small sprayer with about 2 mL of the tea tree oil solution as stated above, and each treated filter paper was placed inside the 9 cm petri dish and 20 to 30 aphids were transferred. The treated filter papers were allowed to dry for 10–15 minutes under the laboratory conditions after spraying with the tea tree solution before the aphids were transferred. In the incubation chamber, petri dishes were placed as described above. After 1, 3, 6, 8 and 24 hrs exposure, mortality was recorded under a microscope. Four experiments with three replicates for each concentration were conducted in the laboratory to determine the activity of the tea tree oils on cotton aphids. To determine the death of cotton aphids, magnifying glass with a needle were used to check the movement of the aphid to assume if dead or not.

Petri dishes containing cotton aphids were weighed before and after the tea tree oil were applied using a weighing scale (Model Number NB-FA110, China).

**Statistical analysis**

ANOVA was performed on mortality rate after the correction for mortality rate by using Abbot Equation (Abbot, 1925), using SPSS software (IBM Corp, Armonk, NY) version 24.0 .

## RESULTS AND DISCUSSION

**Chemical composition of the essential oil**

In the tea tree oil analyzed, 28 chemicals were identified by GC-MS. The most abundant compounds in the tea tree essential oil were Cyclohexane (2.80%), α-pinene (2.27%), (+)-4-Carene (7.31%), p-Cymene (3.8%), D-limonene (1.10%), Eucalyptol (4.98%), γ-Terpinene

(17.74%), Terpinolene (2.78%), (-)-terpinene-4-ol (43.94%) and α-Terpineol (3.61%) (Table 1).

**Table 1.** Constituents identified in tea tree essential oil by using GC-MS

| No. | Compound name | RT * | Percentage (%) of oil composition |
|---|---|---|---|
| 1 | Cyclohexane | 1.97 | 2.8 |
| 2 | α-Thujene | 5.77 | 0.76 |
| 3 | α-pinene | 6.07 | 2.27 |
| 4 | β-pinene | 7.6 | 0.58 |
| 5 | β-Myrcene | 7.94 | 0.55 |
| 6 | α-Phellandrene | 8.72 | 0.28 |
| 7 | (+)-4-Carene | 9.16 | 7.31 |
| 8 | p-Cymene | 9.56 | 3.8 |
| 9 | D-Limonene | 9.73 | 1.1 |
| 10 | Eucalyptol | 9.88 | 4.98 |
| 11 | γ-Terpinene | 11.02 | 17.74 |
| 12 | Terpinolene | 12.47 | 2.78 |
| 13 | (-)-terpinen-4-ol | 18 | 43.94 |
| 14 | α-Terpineol | 18.98 | 3.61 |
| 15 | α-Bisabolol | 25.18 | 0.1 |
| 16 | 4,4-Dimethylpent-2-enal | 26.98 | 0.11 |
| 17 | α-Copaene | 29.77 | 0.1 |
| 18 | α-Gurjunene | 31.67 | 0.31 |
| 19 | Caryophyllene | 32.48 | 0.39 |
| 20 | (+)-Aromadendrene | 33.64 | 1 |
| 21 | Aromadendrene | 34.96 | 0.38 |
| 22 | Isoledene | 35.75 | 0.21 |
| 23 | Viridiflorene | 36.89 | 0.8 |
| 24 | γ-Elemene | 37.18 | 0.22 |
| 25 | δ-Cadinene | 38.59 | 0.87 |
| 26 | Globulol | 42.58 | 0.33 |
| 27 | Ledol | 43.06 | 0.14 |
| 28 | α-Cedrene | 45.01 | 0.16 |

**Contact and Residual toxicity of tea tree oil**

Both contact and residual treatments resulted in the highest mortality rate of *A. gossypii*. The concentration 50 µL/mL had the highest aphid mortality, which was 85.67%, followed by the concentration of 30 µL/mL, then 20 µL/mL, and 10 µL/mL that caused 70.00, 21.67 and 18.33% mortality, compared to untreated aphids after the exposure time 24 hrs (Table 2).

The result showed that the mortality at the concentration of 30 µL/mL was 10.00, 13.33, 21.67, 50.00 and 70.00% at different the exposure time of 1, 3, 6, 8 and 24 hrs, respectively. The use of tea tree oil can be determined as a biopesticide on aphids because it has insecticidal properties. On the other hand, the residual toxicity against *A. gossypii* at the initial screening dose, resulted in 100% mortality at the concentration of 50 µL/mL at 24 hrs exposure time, followed by 92.78% mortality at 30 µL/mL concentration at 24 hrs after treatment (Table 3). While the mortality of cotton aphid *A. gossypii* at the concentration of 1 and 2 µL/mL was less than the high concentration that used.

**Effect of green insecticide formulation in glasshouse**

Table 4 shows the comparison between the effects of tea tree essential oil formulations with chemical pesticide, Karate (Lambda-Cyhalothrin 2.5%) and Levo (Oxymatrine 2.4%). The results indicated that tea tree oil formulations reduced the population of cotton aphids on capsicum plants under glasshouse conditions with somewhat phytotoxicity, which occurred on the capsicum plants. In contrast, both chemical pesticide Karate and botanical insecticide Levo pesticide caused high mortality of cotton aphids *A. gossypii*. Statically, there were no significant differences between tea tree oil formulation, Levo and Karate pesiticides in the mortality of the cotton aphid (*P*-value=0.156 and LSD at 0.05 level=9.023).

**Discussion**

Plant extraction, especially oils, are hypothetically valuable for cotton aphids control. They may be used against various pests and can be sprayed on crops or stored commodities in various methods (Isman, 2000). Tea tree oil was extracted from different parts of the plant, and it has antifeedant, repellent, ovicidal, and insecticidal properties against a variety of insect pests (Isman *et al.*, 2011). Moreover, plant extract oils can be highly effective on insecticide resistant insects

**Table 2.** The effect of tea tree essential oil on the mortality of aphid *Aphis gossypii* after contact treatment

| Tea tree concentration (µL/mL) | µL/m$^2$ | Mortality (%)± SD * | | | | |
|---|---|---|---|---|---|---|
| | | 1h | 3h | 6h | 8h | 24h |
| 0 | 40.62 | 2.67±0.85 | 0.00 | 5.00±5.00 | 1.67±2.89 | 3.33±5.77 |
| 10 | 39.75 | 2.67±2.08 | 4.33±1.15 | 10.67±1.15 | 11.67±2.89 | 18.33±2.89 |
| 20 | 40.06 | 5.00±1.00 | 5.33±1.53 | 11.00±1.00 | 14.33±3.79 | 21.67±7.64 |
| 30 | 39.62 | 10.00±5.00 | 13.33±2.89 | 21.67±2.89 | 50.00±5.00 | 70.00±5.00 |
| 50 | 39.75 | 13.33±2.89 | 18.33±2.89 | 26.67±2.89 | 50.00±5.00 | 85.67±4.04 |

* = Standard division

**Table 3.** The effect of tea tree essential oil on the mortality of aphid *Aphis gossypii* after residual treatment

| Tea tree concentration (µL/mL) | µL/m$^2$ | Mortality (%)± SD * | | | | |
|---|---|---|---|---|---|---|
| | | 1h | 3h | 6h | 8h | 24h |
| 0 | 40.75 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 10 | 40.87 | 3.33±0.00 | 5.56±2.72 | 8.89±2.48 | 11.67±2.88 | 15.00±5.00 |
| 20 | 40.31 | 10.00±3.33 | 11.67±2.88 | 15.00±3.73 | 20.00±3.33 | 24.44±2.72 |
| 30 | 40.75 | 48.33±8.98 | 58.33±5.00 | 62.23±5.67 | 78.34±6.87 | 92.78±2.14 |
| 50 | 40.62 | 69.44±6.51 | 75.00±8.66 | 73.34±7.46 | 88.89±4.29 | 100.00±0.00 |

* = Standard division

**Table 4.** The average of cotton aphid *Aphis gossypii* on capsicum plants after tea tree formulation compared with commercial products in the glasshouse

| Treatments | Concentration (mL/L) | Mortality (%)/day± SD * | | | *P*-value |
|---|---|---|---|---|---|
| | | 2 | 3 | 7 | |
| Control | 0 | 0 | 0 | 0 | 0.000 |
| Levo | 1.5 | 6.30±3.30 | 4.70±2.52 | 0.23±0.25 | 0.033 |
| Tea tree formulation | 5 | 12.50±3.04 | 5.80±4.30 | 2.79±0.99 | 0.052 |
| karate | 0.5 | 7.84±1.44 | 5.78±0.58 | 1.26±2.19 | 0.001 |

* = Standard division

(Farajzadeh *et al.,* 2014). In this study, the influences of tea tree oil varied according to the time of exposure and dose on *A. gossypii*.

The GC-MS investigation (Table 1) revealed variations in tea tree oil components and the percentage of oil content. The top chemicals identified in tea tree oil, according to GC-MS analysis, are (–)-terpinene-4-ol and -Terpinene. Insecticidal characteristics and effects on insect enzymes such as AChE, GST, and CarE are found in these two primary constitutions (Liao *et al.*, 2017).

The findings of this investigation indicated that tea tree oil has insecticidal effects on cotton aphids at varying doses, despite having substantial aphidicidal action against the cotton aphid in varied mortality rates dependent on the concentration of tea tree oil. There were variations in the insecticidal effects of tea tree oil, especially with high tested dosages over 24 hrs exposure period. According to Yazdgerdian *et al.* (2015) and Hollingsworth (2005), the main composition of tea tree oil and the concentration of each ingredient present affect its efficacy on aphids. Significant components α-Pinene, limonene, and camphene have been shown to have aphicidal activities against two species of aphids, which were the woolly beech aphid *Phyllaphisfagi* and the palm aphid *Cerataphis brasiliensis*. Several studies have shown that the component of limonene may attract natural enemies to the infestation of plants by aphids; therefore, plant extract oils with a high quantity of the compound of limonene could function as parasitoids and predator attractants (Yazdgerdian *et al.,* 2015). Other main components identified in tea tree oil, for instance, γ-Terpinene, α-Phellandrene, 4-carene, and terpinene-4-ol own toxic properties that are used on various insect pests (Abtew *et al.*, 2015). The mortality of cotton aphids were 85.67% of the time after being exposed to tea tree oil. Cloyd *et al.* (2009) found similar results, with nearly 100% mortality induced by tea tree oil against rose-grain aphid. Furthermore, applying tea tree oil as a residual application leads to 100% mortality. Our findings are consistent with those of Digilio *et al.* (2008) who achieved significant mortality rates on two species, which were *A. gossypii* and *A. pisum* using various plant oil concentrations.

The findings revealed that a tea tree oil spray formulation tested on capsicum seedlings was effective against cotton aphids. Although the tea tree oil formulation possesses insecticidal properties and is popular for aphid control, the spray application caused phytotoxicity slightly on plant seedlings. According to Digilio *et al.* (2008), many essential oils may affect cotton aphids, although phytotoxicity can occur with particular plant extract oils.

In conclusion, the current study shows that tea tree oil has high toxic effects against aphids, with efficient insecticidal activity in contact and persistent toxicity on cotton aphids. Tea tree oil was shown to be highly efficient against aphids when used as a contact application. On aphids, the residual treatment had the highest aphid mortality. The varied quantities of chemicals that might be utilized in pest management were discovered among the recognized components in tea tree oil. Tea tree oil is a natural plant product that contains a complex combination of chemicals, giving it a wide range of insecticidal and aphicidal effects and there were no significant differences between tested chemical pesticides and tea tree formulations. Although sweet capsicum plants were somewhat impacted, aphids were killed with a green pesticide mixture produced from tested tea tree oil. As a consequence, we recommend that the constituents of the plant extract oils under investigation be assessed as natural insecticides or utilized in the chemical synthesis of a new type of pesticide based on oils and their constituents.

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