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# Insecticidal activity of peels oil of *Citrus sinensis* and summer oil against two scale insects *Aulacaspis tubercularis* (Hemiptera: Diaspididae) and *Milviscutulus mangiferae* (Hemiptera: Coccidae)

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**Abstract:**

Essential oil extracted from peels of balady orange (*Citrus sinensis* L.) was tested for its insecticidal activity at three different concentrations (1000, 5000 and 10000 ppm) against nymphs, adults and gravid females of white mango scale *Aulacaspis tubercularis* (Newstead) (Hemiptera: Diaspididae) and mango soft scale *Milviscutulus mangiferae* (Green) (Hemiptera: Coccidae). Formulated oil of balady orange was bioassayed against the two scale insects and the results revealed that, the formulated oil of balady orange achieved high toxicity against nymphs, adults and gravid females of both *A. tubercularis* and *M. mangiferae* at all used concentrations. Balady orange oil concentrations (0.1, 0.5 and 1%) were more potent against the two scale insects than the reference product (summer oil 1.5%). The essential oil of balady orange was isolated by hydrodistillation and the analysis of gas chromatography–mass spectrometry (GC/MS) revealed the presence of 12 peaks, approximately all peaks were identified. The chemical composition showed that limonene was the main constituent in citrus oil (83.28%). The results of the present study suggested that, formulated balady orange oil used as safe, potential natural products for control of *A. tubercularis* and *M. mangiferae* on mango trees and may be used as alternatives to the reference products after application of these results in the semifield and field experiments.

## Introduction

Mango trees are considered one of the most popular and economic fruit trees in Egypt. They occupy the third rank from the commercial point of view. Mango trees are liable to be infested with many serious pests during their growth stages including white mango scale *Aulacaspis tubercularis* (Newstead)(Hemiptera, Diaspididae) and *Milviscutulus mangiferae* (Green)(Hemiptera: Coccidae). The white mango scale insect *A. tubercularis* injures mangoes by feeding on the plant sap through leaves, branches and fruits, causing defoliation, drying up of young twigs, poor blossoming and so affecting the commercial value of fruits and their export potential especially to late cultivars where it causes conspicuous pink blemishes around the feeding sites of the scales. *A. tubercularis* is a tropical species that may have originated in Asia. It has been recorded mainly from hosts belonging to four plant families: Palmaceae, Lauraceae, Rutaceae, Anacardiaceae, particularly on mangoes and cinnamon (Borchsenius, 1966).

The mango shield scale or mango soft scale *M. mangiferae* a serious pest of mango trees in various parts of the world, is reported on *Mangifera indica* in Egypt which represents the first record of this species in the country (Abd-Rabou and Evans, 2017). In general, continuous and heavy use of the synthetic pesticides has created serious problems such as environmental pollution, toxicity to non-target organisms (parasitoids and predators), pest resistance and pesticide residues (Mohan and Fields, 2002). Therefore, there is an urgent need to develop new, convenient and safer alternatives to synthetic pesticides. Essential oils and their major constituents, attracted research attention in recent years as potential alternatives to synthetic insecticides.

The genus *Citrus* includes several important fruits such as oranges, mandarins, lime, lemons and grapefruits. The essential oils of some citrus species have been reported to have insecticidal properties against insect pests (Elhag, 2000). The major active component of citrus oil is limonene and using 1% limonene mixture was safe for most plants and provided good control of mealybugs and scale insects (Hollingsworth, 2005).

The present study aimed to investigate the efficacy of Egyptian citrus peel essential oil of balady orange (*C. sinensis*), belonging to Family Rutaceae against nymphs, adults and gravid females of both scale insects *A. tubercularis* and *M. mangiferae* on mango trees compared with the summer mineral oil 1.5%. Also, extraction, determination and chemical analysis of essential oil were studied.

## Materials and methods

### 1. Tested citrus species:

The experimental citrus species, balady orange was selected for this study. This citrus species was obtained from a private citrus orchard.

### 2. Insects source:

Infested leaves with both *A. tubercularis* and *M. mangiferae* for laboratory experiments were collected from the Agricultural Experimental Station at El-Kanater El-Khayria, Qalubiya Governorate. Samples were collected randomly from each of the four cardinal directions (East, West, North and South). Leaves were packed in plastic bags and transferred immediately to the laboratory. *A. tubercularis* and *M. mangiferae* were identified by Department of Mealybug and Scale insects, Plant Protection Researches Institute, Agriculture Researches Center. The identification was assured by the aid of Dr. Jean François German in the laboratory of Anses, Laboratoire de la santè des vègètaux, CBGP, Campus Internationalde Baillarguet, France (Attia *et al.,* 2018).

### 3. Extraction of citrus oil:

Citrus oil was extracted by Cavalcanti *et al*. (2004). The essential oil was extracted from the fresh peels (200g weight and 400 ml of distilled water) by hydrodistillation using a modified Clevenger type apparatus for 4 h. The distilled was extracted with diethyl ether after saturation with sodium chloride. The extracted oil was dried over anhydrous sodium sulfate, then packed in dark container and stored at 4°C until used for gas chromatography–mass spectrometry (GC-MS) analysis and bioassays.

### 4. Chemical analysis of essential oil:

#### 4.1. Chemical analysis of citrus peel oil constituents:

The extracted citrus oil was subjected to GC/MS analysis using Shimadzu GC/MS–QP-5050A. Column: DB5, 30m, 0.53mm ID, 1.5µm film. Carrier gas: Helium (flow rate 1.2 ml/min.). Ionization mode: (70ev). The injection volume was 0.5µl (split ratio of 1:100), Temperature program: $50^oC$ (static for 2 min) with gradually increasing (a rate of $4^oC$/ min.) up to $200^oC$ then ($10^oC$/ min.) to

280$^0$C. The detector temperature was 290$^0$C, while, the injector temperature was 250$^0$C.

**4.2. Identification of the chemical constituents:**

Qualitative identification of the essential oil was achieved by library searched data base Willey 229 LIB as well as by comparing their retention indices and mass fragmentation patterns with those of the available references and with published data, (Adams, 2007). The percentage composition of components of the volatile was determined by computerized peak area measurements.

**5. Preparation of formulated orange essential oil:**

Concentrations (1000, 5000 and 10000 ppm) and (0.1, 0.5 and 1%) of formulated oil of balady orange were prepared by emulsifier (Triton-100).

**6. Toxicity bioassays:**

Laboratory bioassays were conducted to determine the bioactivity of formulated citrus oil of balady orange and summer oil against nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae*. The toxicity bioassay was conducted to evaluate toxicity of formulated citrus oil of balady orange to nymphs, adults and gravid females of the two scale insects *A. tubercularis* and *M. mangiferae* at three different concentrations of formulated citrus oil and summer oil (1.5%). Leaves were sprayed with 1ml for five seconds of the formulated citrus oil and summer oil, then, kept at room temperature until the leaves dry. Control insects were sprayed with Triton-x100 emulsifier alone (without oil). Three replicates were used and the experiment was repeated for three times and mortality was recorded after 1, 3 and 6 days

**7. Statistical analysis**

The percentages of mortality in population were calculated by using Stafford and Summers equation (1963) and corrected with Abbot Formula (Abbott, 1925). Data of all experiments were evaluated statistically using ANOVA and means compared using Duncan's Multiple Range Test at $P<0.05$). All statistical analyses were done using the software package Costat program.

**Results and discussion**

**1. Toxicity bioassay:**

The obtained formulated citrus oil of balady orange in this study was mainly conducted to investigate a relationship between the oil constituents and their potency towards nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae* compared to the reference product mineral oil (summer oil).

**1.1. Toxicity of formulated orange oil and summer oil against *Aulacaspis tubercularis*:**

The results of toxicity assays as represented in Table (1), showed that, essential oil of citrus peel exhibited toxicity rate with concentration and time dependent. Formulated peel essential oil achieved high mortality percentages against nymphs, adults and gravid females at the three different concentrations (1000, 5000 and 10000 ppm). The highest toxicity rates against nymphs, adults and gravid females were 90.00±0.0, 88.80±0.92 and 86.93±0.31%, respectively, at the maximum concentration 10000 ppm and after 6 days of treatment. The percentages of mortality achieved by summer oil (1.5%) were 84.59±2.45, 67.50±8.97 and 58.65±9.00% respectively, after 6 days of treatment.

**1.2.Toxicity of formulated orange oil and summer oil against *Milviscutulus mangiferae* :**

The results of toxicity assays as represented in Table (2), showed that, essential oil of citrus peels exhibited toxicity rate with concentration and time dependent. Formulated peel essential oil achieved high mortality percentages against nymphs, adults and gravid females at the different concentrations (1000, 5000 and 10000 ppm) than summer oil (1.5%). The highest toxicity rates of formulated citrus oil nymphs, adults and gravid females were 87.43±0.19, 85.48±1.97and 90.00±0%, respectively, at the maximum concentration 10000 ppm and after

6 days of treatment. While, the percentages of mortality of summer oil (1.5%) were 75.86±2.34, 73.83±3.00 and 80.97±0.95%, respectively, after 6 days of treatment.

The lowest mortality with the two scale insects was obtained with the lowest concentration (1000 ppm) and after one day of assay. The formulated citrus oil and summer oil were more potent against nymphs and adults than gravid females of *A. tubercularis* with the three treatments (after 1, 3 and 6 days).While, the citrus oil and summer oil were more potent against gravid females than nymphs and adults of *M. mangiferae* with the same treatments (1, 3 and 6 days). Generally, balady orange oil concentrations were more potent against the two scale insects than the reference product (summer oil 1.5%). There were significant differences in mortality between control and treated variants (P< 0.05).

**Table (1): Toxic effect orange peel oil *Citrus sinensis* var balady and summer oil against nymphs, adults and gravid females of *Aulacaspis tubercularis* at different concentrations.**

| **Conc. (ppm)** | **Corrected mortality(%)±SD** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Nymphs** | | | **Adults** | | | **Gravid females** | | |
| | 1 day | 3 days | 6 days | 1 day | 3 days | 6 days | 1 day | 3 days | 6 days |
| **1000** | 87.94 ±0.83$^{a}$ | 89.91 ±006 $^{a}$ | 89.64 ±0.24$^{a}$ | 69.49 ±13.11$^{c}$ | 82.48 ±9.88$^{a}$ | 86.75 ±1.26$^{a}$ | 57.72 ±15.64$^{b}$ | 77.39 ±9.74$^{a}$ | 85.51 ±0.99$^{a}$ |
| **5000** | 89.57 ±0.23$^{a}$ | 89.14 ±0.60$^{a}$ | 89.85 ±0.08$^{a}$ | 74.68 ±6.94$^{b}$ | 86.48 ±4.80$^{a}$ | 88.01 ±2.55$^{a}$ | 68.14 ±3.32$^{a}$ | 77.88 ±7.03$^{a}$ | 85.18 ±1.15$^{a}$ |
| **10000** | 89.79 ±0.22$^{a}$ | 89.06 ±0.00$^{a}$ | 90.00 ±0.0$^{a}$ | 78.87 ±7.02$^{a}$ | 86.26 ±6.37$^{b}$ | 88.80 ±0.92$^{a}$ | 68.32 ±11.30$^{a}$ | 80.13 ±8.37$^{a}$ | 86.93 ±0.31$^{a}$ |
| **S.O (1.5%)** | 60.09 ±9.34$^{b}$ | 76.20 ±7.22$^{b}$ | 84.59 ±2.45$^{b}$ | 54.78 ±9.55$^{d}$ | 55.05 ±9.46$^{c}$ | 67.50 ±8.97$^{b}$ | 38.80 ±12.35$^{c}$ | 47.37 ±8.94$^{b}$ | 58.65 ±9.00$^{b}$ |
| **Control** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **F value** | 10.79 ** | 0.32 Ns | 0.83 ns | 2.76 *** | 10.72 ** | 12.394 ** | 23.11 *** | 29.52 *** | 22.30 *** |
| **$LSD_{0.05}$** | 9.14 | 9.43 | 9.41 | 1.995 | 9.66 | 9.15 | 1.4 | 9.02 | 9.43 |
| **S.O= Summer oil** | | | | | | | | | |

**Table (2): Toxic effect of orange peel oil *Citrus sinensis* var balady and summer oil against nymphs, adults and gravid females of *Milviscutulus mangiferae* at different concentrations.**

| **Conc. (ppm)** | **Corrected mortality(%)±SD** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Nymphs** | | | **Adults** | | | **Gravid females** | | |
| | **1 day** | **3 days** | **6 days** | **1 day** | **3 days** | **6 days** | **1 day** | **3 days** | **6 days** |
| **1000** | 69.96 ±0.90$^{b}$ | 87.43 ±0.28$^{a}$ | 86.74 ±0.46$^{a}$ | 55.84 ±3.93$^{c}$ | 78.25 ±0.63$^{a}$ | 80.96 ±0.80$^{a}$ | 78.85 ±0.50$^{b}$ | 89.09 ±0.50$^{a}$ | 88.63 ±0.00$^{a}$ |
| **5000** | 79.02 ±0.13$^{ab}$ | 86.57 ±0.51$^{a}$ | 87.43 ±0.29$^{a}$ | 71.55 ±4.48$^{b}$ | 80.96 ±0.86$^{a}$ | 80.96 ±0.00$^{a}$ | 89.09 ±0.00$^{a}$ | 90.00 ±0.00$^{a}$ | 90.00 ±0.00$^{a}$ |
| **10000** | 81.77 ±0.07$^{a}$ | 87.11 ±0.33$^{a}$ | 87.43 ±0.19$^{a}$ | 81.46 ±10.38$^{a}$ | 83.22 ±6.93$^{a}$ | 85.48 ±1.97$^{a}$ | 88.63 ±2.51$^{a}$ | 89.09 ±0.00$^{a}$ | 90.00 ±0.00$^{a}$ |
| **S.O (1.5%)** | 48.48 ±7.32$^{c}$ | 58.93 ±6.98$^{b}$ | 75.86 ±2.34$^{a}$ | 44.85 ±6.55$^{d}$ | 58.93 ±3.67$^{b}$ | 73.83 ±3.00$^{a}$ | 50.65 ±4.45$^{c}$ | 77.02 ±1.29$^{a}$ | 80.97 ±0.95$^{a}$ |
| **Control** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **F value** | 27.31 *** | 24.58 *** | 1.10 Ns | 21.90 *** | 7.41 * | 1.13 ns | 20.72 *** | 1.52 ns | 1.29 ns |
| **$LSD_{0.05}$** | 9.41 | 9.41 | 17.73 | 11.28 | 13.38 | 15.06 | 12.94 | 16.41 | 12.45 |
| S.O= **S.O=Summer oil** | | | | | | | | | |

Pumnuan *et al.* (2015) showed that, fresh peels essential oils of four citrus species recorded moderate toxicity at 2ml/L air (fumigation) and high toxicity at 2ml/L air against larvae of mealybug mealybug *Pseudococcus jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae) at 24h. These findings are confirmed by Karamaouna *et al*. (2013), they showed that the citrus peel essential oils of lemon (*Citrus limon*) and balady orange (*C. sinensis*) were the most toxic of all the tested essential oils against 3rd instar nymphs and females adults of the vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae) . El-Badawy (2015) found that, all tested citrus oils especially balady orange achieved high insecticidal and repellent activities against mealybug *Icerya seychellarum* (Westwood) (Hemiptera: Monophlebidae).

**1.3.Comparison of total mean mortality of citrus oil and summer oil against *Aulacaspis tubercularis*.**

From the data of total mean values presented in Tables (3 and 4) it could be demonstrated that, at the three different concentrations (0.1, 0.5 and 1.0%) the formulated peel citrus oil was more nymphscidal and adultscidal effect than the summer oil (1.5%) against *A. tubercularis* and *M. mangiferae* after 1, 3 and 6 days of assay. The recorded total mean mortality values (69.62, 83.16 and 87.17%) of the lowest concentration (0.1%) of orange oil were higher than summer oil (1.5%) mortality values (52.46, 61.04 and 71.52%) against *A. tubercularis* after 1, 3 and 6 days of assay, respectively. The recorded total mean mortality values of the formulated orange oil at the other two concentrations (0.5 and 1.0%) against *A. tubercularis* were (77.17 and 77.62 %), (84.05 and 82.14%) and (87.21and 88.24 %), respectively, after the same time (Table, 3).

The mortality percentages values of the citrus oil against *M. mangiferae* after one day of assay ranged from 65.68 (conc. 0.1%) to 86.62% (conc. 1%), and after three days ranged from 87.09 to 95.68%, while, after six days ranged from 87.77 to 98.33%, respectively. Also, the lowest concentration (0.1%) of orange oil was higher than summer oil (1.5%) mortality values against *M. mangiferae* after the same time (Table, 4). The variation of the mortality values of citrus oil against *A. tubercularis* and *M. mangiferae* depending on the toxicity of the formulation of citrus oil, the mealybug and scale insect life stage.

These findings are confirmed by Karamaouna *et al*. (2013), who showed that, the $LC_{50}$ values of citrus (*C. sinensis* and *C. limon*) oils ranged from 2.7 to 8.1mg/ml depending on the essential oil and the mealybug life stage. These $LC_{50}$ values were significantly lower than the $LC_{50}$ of the reference paraffin oil in the respective *P. ficus* life stages. Results of El-Badawy (2015), revealed that, the oil of balady orange achieved the highest toxicity against nymphs, adults and gravid females of mealybug *I. seychellarum*.

**Table (3): Toxic effect comparison of *Citrus sinensis* var balady oil and summer oil against total mean of nymphs, adults and gravid female of *Aulacaspis tubercularis* at different concentrations.**

| Time (days) | Corrected mortality (%) | | | |
|---|---|---|---|---|
| | Conc. (%) | | | Summer oil (1.5%) |
| | 0.10 | 0.50 | 1.00 | |
| | Total Mean | | | Total Mean |
| 1 day | $69.62^{c}$ | $77.17^{c}$ | $77.62^{c}$ | $52.46^{c}$ |
| 3 days | $83.16^{ab}$ | $84.05^{b}$ | $82.14^{b}$ | $61.04^{b}$ |
| 6 days | $87.17^{a}$ | $87.21^{a}$ | $88.24^{a}$ | $71.52^{a}$ |
| F value | $3.54^{ns}$ | $0.54^{ns}$ | 18.95* | 9.535* |
| $LSD_{0.05}$ | 16.91 | 19.02 | 18.95 | 10.69 |
| Total Mean= Total mean of nymph, adult and gravid female | | | | |

**Table (4): Comparison of toxic effect of balady orange peels oil and summer oil against nymphs, adults and Gravid females of *Milviscutulus mangiferae* at different concentrations**

| Time (days) | Corrected mortality(%) Conc. % 0.10 | 0.50 | 1.00 | Summer oil (1.5%) |
|---|---|---|---|---|
| | Total Mean | | | Total Mean |
| **1 day** | 65.68[b] | 76.73b[a] | 86.62[a] | 37.15[c] |
| **3 days** | 87.09[a] | 87.37[a] | 95.68[a] | 50.28[b] |
| **6 days** | 87.77[a] | 88.35[a] | 98.33[a] | 65.40[a] |
| **F value** | 11.272** | 3.851[ns] | 1.54[ns] | 14.39** |
| **$LSD_{0.05}$** | 12.95 | 11.36 | 17.11 | 12.90 |

## 2. Chemical analysis of citrus peel essential oil:

The essential oil yield of fresh citrus peels of *C. sinensis* was (4.30%). The chemical compositions of the essential oil of citrus peels are presented in Table (5). The essential oil analysis by GC/MS revealed that, the presence of 12 peaks, all peaks were identified, representing 99.70 % of the essential oil of balady orange. The major constituents of this essential oil mainly belonged to two groups: monoterpene and oxygenated monoterpenes. Oxygenated monoterpenes hydrocarbons with contribution of 8.33% constituted the second major portion of the essential oil after monoterpenes hydrocarbons (89.84%) from peel oil. The chemical analysis of the citrus oil showed limonene as the main constituent (83.28%) for balady orange. The most abundant ingredients beside to limonene, were linalool (3.97%), β-myrcene (3.63%), β-Citral (1.97%), p-Cymene (1.73%) , α-Citral (1.64%) and Linalyl acetate (1.56%) in the citrus peels oil. The monoterpene hydrocarbons *α*-pinene, β-pinene and γ-Terpinene are present. Overall results indicated that the toxic effects of citrus oil balady orange on *A. tubercularis* and *M. mangiferae* could be related to the high content of limonene.

**Table (5): Chemical composition of essential oil from balady orange peels .**

| No. | Components | RT(min.) | Ratio (%) |
|---|---|---|---|
| **1** | α-thujene | 7.125 | 0.05 |
| **2** | α-Pinene | 8.718 | 1.34 |
| **3** | β-Pinene | 10.14 | 0.29 |
| **4** | β-Myrcene | 10.718 | 3.63 |
| **5** | α-Terpinene | 11.84 | 0.20 |
| **6** | D-Limonene | 12.343 | 83.28 |
| **7** | γ-Terpinene | 12.125 | 0.22 |
| **7** | p-Cymene | 12.163 | 1.73 |
| **8** | β-Linalool | 12.952 | 3.97 |
| **9** | Linalyl acetate | 13.066 | 1.56 |
| **10** | α-cis-Citral | 13.234 | 1.64 |
| **11** | β-cis-Citral | 14.477 | 1.97 |
| **12** | Geraniol | 14.739 | 0.69 |
| | Monoterpene Hydrocarbons | - | 89.84 |
| | Oxygenated Monoterpene Hydrocarbons | - | 8.33 |
| | Other compounds | | 1.53 |
| | Total | - | 99.70 |

Our results of the chemical composition of citrus peel oil are in agreement with many other studies (Ahmad *et al*., 2006, Asekun *et al*., 2007 and El-Badawy, 2015). All these studies showed that, limonene was the main component with high variation in all citrus peel oils and also, there are considerable variations in the other constituents of the chemical composition of citrus oils. Such variation in chemical composition (Limonene content and other constituents) in citrus peel oils may be related to the time of harvesting, the degree of freshness, genetic makeup and the size of the fruit. Also, geographical location, fruit variety and method of extraction (Ahmad *et al*., 2006).

Regarding to potency of citrus oil against nymphs, adults and gravid females of *A. tubercularis* and *M. mangiferae*, the data presented in Tables (1-4) indicated that the potency of the tested formulated oil was related to the major component limonene content of that oil. These results are confirmed by El-Badawy (2015), who showed that the toxic effect of five citrus oils on *I. seychellarum* could be related to the high content of limonene. Also, these results are in agreement with these obtained who showed that by Hollingsworth (2005). The best limonene mixture (1% limonene, 0.75% emulsifier APSA-80 and 0.1% surfactant Silwet) controlled from 69 to 100% of mealybugs and scale insects, depending on the species, insect stage and application method.

Citrus oil of balady orange was more toxic than summer oil against *A. tubercularis* and *M. mangiferae*, so, it can be used as an effective natural alternative to mineral oil (summer oil). It is recommended to expand such laboratory experiments to semifield and field conditions and determine the efficacy of balady orange oil against *A. tubercularis* and *M. mangiferae* and other mealybug and scale insect species.

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