Research Article

Efficacy of some Plant Essential Oils as Green Insecticides to Control Whitefly, *Bemisia tabaci* (Gennadius)

Heba Youssif El-Sayed Ibrahim* and Mohamed El-Hoseiny Mostafa

Plant Protection Research Institute, Agriculture Research Center (ARC), Dokki, Giza, 12618, Egypt.

Ten plant essential oils were extracted and then tested for their activity as natural insecticides against the whitefly, *Bemisia tabaci* (Gennadius). *In-vitro* bioassay, the contact toxicity of the tested essential oils to eggs and 3rd instar nymphs was determined. The most effective of tested essential oils as ovicides were *Artemisia absinthium*, *Cyperus articulates*, and *Thyme vulgaris* with LC50: 0.157, 0.305 and 0.334 ppm, respectively. Also, the most effective oils against 3rd instar nymphs were *A. absinthium* followed by *C. articulates*, and *T. vulgaris* with LC50: 7.268, 7.865 and 8.989 ppm respectively. Repellency effect and oviposition deterrence of the tested essential oils were studied through choice and no-choice tests. The most repellents and anti-oviposition oils were *A. absinthium*, *T. vulgaris*, *C. articulates* and *Pluchea dioscoridis* in both choice and no-choice tests. Also, the efficacy of the most effective oils in the laboratorial experiments were tested against *B. tabaci* in open field conditions. The most efficient one was *A. absinthium* which showed great reduction percentage of *B. tabaci* populations (87.6%), followed by *C. articulates* (85.0%), *T. vulgaris* (81.9%), *Mentha longifolia* (78.6%) then *Syzygium aromaticum* (51.7%).

Keywords: *Bemisia tabaci*, Essential oils, contact toxicity, repellency and oviposition deterrenncy.

INTRODUCTION

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous and multivoltine insect pest in the field and greenhouses (Oliveira et al., 2001) with great economic impacts on many crops such as cotton, vegetables, fruit crops and ornamentals (Gerling, 1990 and Omid Bakhsh et al., 2010). Whiteflies damage plants directly by sucking plant sap causing the silvering of leaves, irregular colour of fruits and growth stunting especially in young plants. And indirectly, whiteflies transmit several plant viruses (Lapidot and Polston, 2006). *B. tabaci* transmits plant viruses in seven distinct groups including: potyviruses, geminiviruses, carlaviruses, closteroviruses, nepoviruses, luteoviruses and DNA-containing rod-shaped virus (Thompson, 2011). Also, they excrete honeydew which stimulate the growth of sooty mold hindering the photosynthesis process (Byrne and Bellows, 1991).

The excessive use of synthetic chemical insecticide led to many environmental problems, besides development of insect resistance with subsequent population outbreaks (Palumbo et al., 2001). Therefore, it became necessary to seek for secure alternatives. Some plant derived compounds showed promising efficiency against insecticide-resistant insect pests (Ahn et al., 1997). Essential oils play an important role as safe alternatives having insecticidal activity against wide spectrum of insect pests. They may act as repellents (Park et al., 2006, Nagassoum et al., 2007 and Abd-Elhady, 2012), antifeedants (Isman, 2002 and Hernandez-Lambrano et al., 2014), molting and growth inhibitors (Athanassiou et al. 2014 and Aziza et al., 2014), fecundity inhibitors or toxins (Enan, 2001; Isman, 2006; and Baldwin et al., 2013). This study aimed to test the efficacy of ten essential oils extracted from local Egyptian available plants against different stages of *B. tabaci*. The repellency effects of female settlement and oviposition was also assessed.

*Corresponding author*: Heba Youssif El-Sayed Ibrahim, Plant Protection Research Institute, Agriculture Research Center (ARC), Dokki, Giza, 12618, Egypt. Email: hyei_youssif@yahoo.com
MATERIALS and METHODS

Plant Materials and Essential Oils Extraction

The seeds of Artemisia absinthium L. (Asteraceae); tubers of Cyperus articulates L. (Cyperaceae); aerial parts of Anethum graveolens L. (Apiaceae); leaves of Pluchea dioscoridis L. (Asteraceae), Mentha longifolia (L.) Huds. (Lamiaceae), Lantana camara L. (Verbenaceae); and whole plant of Thyme vulgaris L. (Lamiaceae) were collected from Mansoura University farm while roots of Zingiber officinale Rosc. (Zingiberaceae); seeds pods of Elettaria cardamomum maton. (Zingiberaceae); and buds of Syzygium aromaticum (L.) Merr. & Perry (Myrtaceae) were acquired from herbal markets of Mansoura, Egypt. All plant materials were identified at Plant Department, Faculty of Science, Mansoura University. Essential oils of different plant materials were extracted by hydro-distillation for 4 hours in Clevenger-type apparatus. Then they were dried over anhydrous sodium sulphate and kept frozen in dark glass tubes until application. All essential oils were formulated as emulsion in water and 0.1% triton X-100. Four concentrations of each essential oil were tested immediately after preparation.

Pure culture of Whitefly B. tabaci and Host Plants

A pure culture of B. tabaci biotype B was obtained from a colony reared on cabbage, Brassica oleracea var. capitata under glass greenhouse at Faculty of Agriculture, Mansoura Univ. The whitefly strain was transferred to the laboratory and reared on cabbage seedlings planted in small pots 25cm (diameter) and kept under plastic greenhouse conditions of 27±2 °C, 70±5 RH and 14:10 Light: Dark. For the in vitro bioassay experiments, cabbage seeds were grown in plastic pots (15cm diameter) under plastic greenhouse to avoid any insect infestation. The seedlings were allowed to reach 20-25 cm high, with 4-5 fully expanded leaves.

In-vitro Bioassay

Bioassay for contact toxicity

All developmental stages of B. tabaci were obtained by releasing adults (= 100 individuals per plant) on cabbage seedlings free from any insect infestation in plastic cages, and were allowed to deposit eggs. After 24 hours, adults were removed, and the deposited eggs developed to the suitable stages for assessment.

Thirty individuals of the tested developmental stages were counted under dissecting microscope and marked with a water proof pen to be considered as a replicate. Each concentration had three replicates in addition to control which was sprayed with water and 0.1% triton X-100 using a hand sprayer. The sprayed leaves containing marked developmental stages were individually placed in test tubes filled with water to maintain leaves freshness along the experiment, and kept under plastic cages with fine polyester netting side panels for ventilation. All labatorial experiments were conducted in stable conditions of 27±2 °C, 70±5 RH and 14:10 L: D.

Effect of the tested essential oils on B. tabaci eggs

Immediately after adults’ removal, the fresh eggs were counted, then treated with the tested essential oils formulations and observed daily along 10 days until eggs hatching was completed. The ovicidal effect was determined by the number of unhatched eggs.

Effect of the tested essential oils on nymphal stage

When the hatched eggs developed to the third instar stage-nymphs, they were treated with different concentrations of the essential oils. The mortality percentages were calculated after five days. Dried and shrunk brown nymphs were considered as dead.

For contact toxicity, the average of mortality percentages of B. tabaci was estimated and corrected using Abbott’s formula (1925). The corrected mortality percentage of each tested oil was statistically calculated according to Finney (1971). The corresponding concentration probit lines (LC-p lines) were estimated in addition to determination of 50 and 90% mortalities, slope values of the tested oils were also estimated. In addition, the efficiency of different tested fungi was measured by comparing the tested oils with the most effective one by using Sun’s equation (1950).

Bioassay for repellency effect and oviposition deterrency

Choice test

Twenty cabbage seedlings with three leaves were divided into two equal groups. Each plant of the first group was separately sprayed with one of the tested essential oils formulations and the other group was sprayed only with water and triton X-100 as a control. The repellency effect of the essential oils was tested at concentration of LC90 of nymphal stage. Each treated plant was placed with another untreated one (control) in a net cage (60cm x 60cm x 60cm) at 30cm distance of each other. After an hour of treatment, 100 adult whiteflies were released into the center of the cage floor. The numbers of whiteflies settled on both sides of leaves were counted at 12, 24 and 48 hours after release. Also, the eggs deposited on leaves were counted under dissecting microscope after 48 hrs. of release. The experiment was repeated three times (three replicates) under the same conditions of 27±2 °C, 70±5 RH and 14:10 L: D photoperiods. Repellency index (Baldin and Lara 2001; Baldin et al., 2013 and Schlick-Souza et al., 2011) was calculated according to the equation: RI=2T / (T+C), where T is the number of insects on treated plants and C is the number of insects on the control plant. When RI values <1, it indicated repellence of B. tabaci by treated plant compared with the control; RI>1, that indicated attractiveness of B. tabaci to the treated plant compared with the control.
Also, oviposition deterrence index (ODI) (Hang et al. 1982) was calculated according to the equation: ODI= [(T-C)/(T+C)] x 100, where T is the number of eggs counted on the treated plants, and C is the number of eggs counted on the control plants. ODI values vary from +100 (very attractive) to -100 (complete deterrence). RLs and ODIs values of choice and no-choice test were separately analyzed by Two-Way ANOVA using CoStat Software (2004).

No choice test

Cabbage seedlings with three leaves were individually placed in net cages (30 cm × 30 cm × 60 cm), and each cage contained one cabbage plant. Three cages were used as replicates for each oil treatment, in addition to three cages as controls. The treated plants were sprayed with LC$_{90}$ of nymphal stage. Fifty adult whiteflies were released into each cage after an hour of treatment. The adults were counted at 12, 24, and 48 h after release. Also, the eggs deposited were counted under a dissecting microscope at 48 h after the whiteflies release. Both RI and ODI were calculated.

Field Bioassay

The five most efficient oils were tested against B. tabaci on cabbage, B. oleracea var. capitata in open field conditions. The field was divided into equal blocks, and each oil was sprayed on four blocks (four replicates) with invito-LC$_{90}$ of 3rd instar-nymphs in addition to another four replicates as controls. The blocks were in completely random arrangement. Thirty leaves from cabbage seedlings in each replicate were examined directly for counting the adults and under a dissecting microscope for counting the other stages. The effect of the tested oils was observed daily till 3rd day after treatment. Efficiency of the tested essential oils were calculated according to Henderson-Tilton (1955).

RESULTS AND DISCUSSION

Bioassay for contact toxicity

In *vitro* bioassay showed that all the tested oils significantly suppressed the B. tabaci eggs hatchability by contact toxicity. A. absinthium was the most efficient oil followed by C. articulates, T. vulgaris, M. longifolia, S. aromaticum, A. graveolens, E. cardamomum, L. camara, Z. officinal and P. discoridis that showed the lowest activity. LC$_{50}$, LC$_{90}$ values and toxicity index were mentioned in Table 2. Our results about high contact toxicity of T. vulgaris essential oil against B. tabaci biotype B. are agreed with those presented by Yang et al. (2010) and Kim et al., (2011). Also, the present results are in accordance with those presented by Yarahmadi et al. (2013). They illustrated the contact toxicity and significantly population reduction of *Bemisia tabaci* by *Artemisia* essential oil.

Data clearly demonstrated that eggs were found to be more susceptible to the tested essential oils than nymphs. Moreover, the tested essential oils varied in their activity, this may be due to variation of their chemical constituents. Several studies pointed out the neurotoxic action of essential oils by acetylcholinesterase (AChE) inhibition or by blocking the octopamine receptors (Enan 2001). Terpenoids, the major constituents of essential oils and the responsible for their insecticidal toxicity, are highly selective to insects since they are targeted to the insect-selective octopaminergic receptor, a non-mammalian target. Also, essential oils interfere with γ-aminobutyric acid (GABA) gate chloride channels in insects (Priestley et al. 2003).

Bioassay for repellency effect and oviposition deterreny

In choice test, the repellency of the tested essential oils against B. tabaci adults were studied. It was observed that almost all tested essential oils showed repellency effect except two oils, S. aromaticum and L. camara that showed slight attractant effect. There were high significant differences among the tested oils (F: 1330.85; df: 9; p< 0.01).

Taking time after adults introduction into consideration, (Fig. 1- 3), at 12 and 24 hours after adult introduction, A. absinthium showed the highest repellency that followed by C. articulates, T. vulgaris, P. discoridis, M. longifolia, A. graveolens, E. cardamomum then Z. officina which showed slight repellency effect. At 48 hours after adults-introduction, observations showed that the repellency effects of the tested oils decreased to some extent. Also, the attraction effects of S. aromaticum and L. camara decreased to values that were comparable to the control or even less. There were high significant differences among the experimental counting periods (F: 1198.97; df: 2; p< 0.01).

The oviposition deterrency of the tested essential oils was tested against B. tabaci adults at 48 hours after adults-introduction. The oviposition deterrency indices (ODIs) (Fig. 4) indicated that A. absinthium showed high deterrency and oviposition reduction effects (-63.41%) followed by T. vulgaris (-42.58%) and P. discoridis (-34.91%). The other essential oils except S. aromaticum, L. camara and E. cardamomum, showed slight oviposition deterrency whilst S. aromaticum, L. camara and E.
Table 1: Ovicidal effects of plant essential oils against *B. tabaci* eggs after 10 days of treatment.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Eggs</th>
<th>LC₅₀ (ppm) and confidence limits at 95%</th>
<th>LC₉₀ (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>X²</th>
<th>Toxicity index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>0.157</td>
<td>5.753</td>
<td>0.820± 0.186</td>
<td>1.20</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td>0.135</td>
<td>7.640</td>
<td>0.943± 0.198</td>
<td>1.058</td>
<td>47.006</td>
<td></td>
</tr>
<tr>
<td><em>C. articulates</em></td>
<td>0.305</td>
<td>2.776</td>
<td>1.335± 0.277</td>
<td>0.618</td>
<td>51.475</td>
<td></td>
</tr>
<tr>
<td><em>M. longifolia</em></td>
<td>0.452</td>
<td>35.551</td>
<td>0.676± 0.171</td>
<td>3.759</td>
<td>34.735</td>
<td></td>
</tr>
<tr>
<td><em>S. aromaticum</em></td>
<td>0.336</td>
<td>2.311</td>
<td>41.692</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. officinal</em></td>
<td>1.384</td>
<td>4.864</td>
<td>11.925</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. graveolens</em></td>
<td>0.612</td>
<td>44.324</td>
<td>25.654</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. camara</em></td>
<td>1.085</td>
<td>32.453</td>
<td>146.109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. cardamomum</em></td>
<td>0.726</td>
<td>16.711</td>
<td>104.749</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. discoridis</em></td>
<td>7.33</td>
<td>32.453</td>
<td>993.05</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Toxicity of plant essential oils against 3rd instar nymphs of *B. tabaci* after 5 days of treatment.

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>3rd instar</th>
<th>LC₅₀ (ppm) and confidence limits at 95%</th>
<th>LC₉₀ (ppm) and confidence limits at 95%</th>
<th>Slope ± SE</th>
<th>X²</th>
<th>Toxicity index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>7.268</td>
<td>43.856</td>
<td>1.642± 0.390</td>
<td>1.578</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>T. vulgaris</em></td>
<td>3.816</td>
<td>10.343</td>
<td>146.109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. articulates</em></td>
<td>4.998</td>
<td>34.502</td>
<td>92.409</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. longifolia</em></td>
<td>9.491</td>
<td>34.502</td>
<td>92.409</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aromaticum</em></td>
<td>12.825</td>
<td>34.502</td>
<td>92.409</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Z. officinal</em></td>
<td>17.667</td>
<td>88.044</td>
<td>41.139</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. graveolens</em></td>
<td>16.339</td>
<td>118.929</td>
<td>146.109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. camara</em></td>
<td>11.107</td>
<td>25.855</td>
<td>44.483</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. cardamomum</em></td>
<td>15.236</td>
<td>43.502</td>
<td>31.738</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. discoridis</em></td>
<td>22.9</td>
<td>84.225</td>
<td>912.20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*cardamomum* showed slight attractant effects. There were high significant differences among the tested essential oils ODI (F: 357.364; df: 9; p< 0.01). It was clear that there was positive correlation between RI and ODI. This agreed with Silva et al. 2012, who suggested that the repellency effect is a factor of oviposition inhibition.

In no-choice test, all the tested essential oils showed repellency effects and varied in repellency degree, (Fig. 5-7). At 12h after adult’s introduction, *A. absinthium* showed the highest repellency followed by *P. discoridis, T. vulgaris, C. articulates, A. graveolens, M. longifolia, E. cardamomum* then *Z. officinal*, while *S. aromaticum* and *L. camara* hardly
showed slight repellency effect. All repellency effects of the tested essential oils decreased at 24h after adults-introduction and continued decreasing at 48h. It was observed that almost all oils lose their repellency effects at 48 after adults-introduction except A. absinthium, T. vulgaris, C. articulates and P. discoridis. There were significant effects of the essential oils and time (F: 366.63; df: 9; p< 0.01), (F: 694.94; df: 2; p< 0.01), respectively. Previous data showed that repellency (RIs) of the tested essential oils in no-choice test was less than that of choice test. In no-choice test, there was no alternative nutrition source for B. tabaci adults except for the plants treated with essential oils, so, the adults forced themselves to settle on the treated plants for feeding with frequent changing of feeding sites.

All tested essential oils showed oviposition deterrenny against B. tabaci adults except S. aromaticum which showed no effect. As shown in Fig. 8, the highest oviposition deterrenny effect was showed by A. absinthium (-57.93%) followed by T. vulgaris (-38.98%) and P. discoridis (-32.93%). There were high significant differences of the tested essential oils ODI (F: 618.774; df: 9; p< 0.01).
Table 3: Reduction % averages of B. tabaci treated with tested essential oils in open field conditions

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Pre-spray</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Overall Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean No.</td>
<td>% Red.</td>
<td>Mean No.</td>
<td>% Red.</td>
<td>Mean No.</td>
</tr>
<tr>
<td>A. absinthium</td>
<td>592.3a</td>
<td>60.3c</td>
<td>90.3</td>
<td>68.5c</td>
<td>89.3</td>
</tr>
<tr>
<td>C. articulates</td>
<td>493.5a</td>
<td>57.5c</td>
<td>88.9</td>
<td>76.3c</td>
<td>85.7</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>542.3a</td>
<td>80.0c</td>
<td>85.9</td>
<td>99.8c</td>
<td>83.0</td>
</tr>
<tr>
<td>M. longifolia</td>
<td>499.5a</td>
<td>80.5c</td>
<td>84.6</td>
<td>107.3c</td>
<td>80.1</td>
</tr>
<tr>
<td>S. aromaticum</td>
<td>515.3a</td>
<td>326.0b</td>
<td>39.6</td>
<td>212.8a</td>
<td>61.8</td>
</tr>
<tr>
<td>Control</td>
<td>525.8a</td>
<td>550.8a</td>
<td>567.8a</td>
<td>578.0a</td>
<td>565.5a</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>134.9</td>
<td>61.6</td>
<td>40.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Field Bioassay

The field experiment clarified the great suppression of B. tabaci populations by application of essential oils as natural insecticides. The pre-treatment counts recorded an hour before spraying indicated that there was no significant difference in the whitefly populations in all blocks. At the 1st day after treatment, A. absinthium showed the highest population reduction followed by C. articulates, T. vulgaris, M. longifolia, and S. aromaticum with mean reduction %: 90.3, 88.9, 85.9, 84.6 and 39.6%, respectively as shown in Table 3. At the 3rd day after treatment, the population reduction was 83.3, 80.5, 76.9, 71.2 and 53.7%, respectively. The average reduction percentages of the selected essential oils were 87.6, 85.0, 81.9, 78.6 and 51.7%, respectively.

All previous data emphasized that essential oils are promising factors that can act as insecticides, ovicides, repellent and insect-oviposition deterrent. Therefore, essential oils are worthy of further studies in order to develop their application in wide scales as environmentally-friendly insecticides.

CONCLUSION

The present study highlighted the importance of the essential oils as green insecticides. Our results illustrated that A. absinthium, C. articulates and T. vulgaris showed high contact toxicity, repellency effect and oviposition deterreny against the whitefly, B. tabaci. Therefore, they are recommended to be used in wide scales in B. tabaci control.

REFERENCES


