An evaluation of some eco-friendly biopesticides against 
Bemisia tabaci on two greenhouse tomato varieties in Egypt

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Abstract
This study has two main approaches. First, it exploits the susceptibility of tomato cultivars as a prophylactic measure to detect auto resistance characters of the tested tomato varieties against Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae). Secondly, it evaluates the efficacy of different bio-rational insecticides against B. tabaci under greenhouse conditions. The results exhibited a special significance in B. tabaci infestation suitability between the two tomato varieties with a high infestation significance found in the Shifa F1 hybrid tomato variety compared to the Savera F1 hybrid tomato variety in the first plantation period. Subsequently, in the second plantation period, there was a significant difference between the two tomato varieties. Bemisia tabaci showed a preference for the Shifa F1 hybrid over the Savera F1 hybrid tomato variety. These differences occurred during the 1st, 2nd, 4th, 6th, 7th, 8th, and 10th weeks. In the experimental trial for the efficacy of eco-friendly bio-rational insecticides, spinosad, azadirachtin, Beauveria bassiana and Metarhizium anisopliae, there were significant differences between the treated and untreated plants during the two plantation periods. A high efficacy of spinosad on the B. tabaci population was found. Bemisia tabaci infestation under all the applications was reduced from 50 to 94.61% for the two plantation periods. This obvious decrease in B. tabaci population increase attention to benefits of the different bio-rational insecticides.

Key words: Bemisia tabaci, bio-rational insecticides, greenhouse, susceptibility tomato

Introduction

Tomato, Lycopersicon esculentum Mill. is a very important vegetable crop throughout the world. Annually 107 million metric tons are produced, with fresh market tomatoes making up to 72% of the total (FAOSTAT 2002). Tomatoes are grown both under plastic covered greenhouses and in open fields. Tomatoes, wherever grown, are hosts for many kinds of insect pests: whiteflies, aphids, cabbage loppers, cutworms, tomato leaf miners, tomato fruit worms, tomato pinworm, mealy bugs and flea beetles (Abd El-Ghany 2011; Ibrahim et al. 2015). The whitefly Bemisia tabaci (Gennadius) (Homoptera: Aleyrodidae), a cosmopolitan insect pest, is one of the most destructive agricultural pests worldwide owing to its ability to feed on hundreds of plant species, many of which are important agricultural crops (Brown 2010; Dinsdale et al. 2010). Whiteflies are now globally distributed and are serious pests of cultivated crops in tropical and subtropical areas including Africa, Asia, Central America, South America, and the West Indies (Cock 1986; Chu et al. 2007). They are widespread throughout the Mediterranean region and have become increasingly important pests of cotton and vegetables in Egypt (De Barro et al. 2011).

The whitefly is a polyphagous insect pest on more than 600 different plant species (Oliveira et al. 2001; Bayhan et al. 2006; Stansly and Natwick 2010). It causes economic losses in vegetable, fiber, and ornamental crops due to both direct damage through phloem
feeding and injection of toxins and indirect damage to the host plant through its ability to transmit plant viruses (Pereira et al. 2004; Brown 2010). The direct damage elicited by B. tabaci has a vast impact on plant health and consequently yield. The indirect damage caused by the whitefly is even more destructive for agriculture (Lima et al. 2000). Indirect damage occurs through transmission of geminiviruses; whiteflies are vector of more than 300 plant viruses (Hogenhout et al. 2008). Moreover, an unfavorable side effect of whitefly infestation is the production of carbohydrate-rich honeydew excretions, which make the leaves sticky, impair photosynthesis and support the growth of sooty mold fungi on the plant leaf and fruit surface (Stansly and Natwick 2010). Whiteflies have a high level of resistance to chemical pesticides (Horowitz et al. 2005). Therefore, the present work aims to exploit the susceptibility of tomato cultivars as a prophylactic measure to its resistance characters against B. tabaci under greenhouse conditions. Moreover, the efficacy of different bio-rational insecticides against B. tabaci was evaluated.

Materials and Methods

Tomato varieties and plantation

The work was carried out in the greenhouse unit of the National Research Center (NRC) farm at El-Behira Governorate, District of Kom Hamada, about 160 km from Cairo. The farm covers 126 ha, of which about 7 ha make up a contiguous plot of greenhouses for vegetable crops. The average annual temperature of the area is around ±21.2°C, relative humidity (RH) around 57% and rainfall around 11.6 (±4.5). The soil is sandy, deep, and non-saline or slightly saline (EC values range between 0.2 and 0.5 dS · m⁻¹). Field capacity and wilting point are low (8–9 and 2–3% of soil moisture content, respectively). Soil fertility has a low content of macro- and micronutrients and organic matter. Sprinkler and drip irrigation systems are used to irrigate field crops, vegetables and fruit trees. The principal source of irrigation water for the whole area is the Nubaria canal. The greenhouse is oriented north to south, and is covered by polyethylene plastic. The total area of the greenhouse is 480 m² (60 × 8 m). The soil was mixed with chick manure and tilled 3 times, once a week to increase fertility and reduce the viability of weed seeds and other possible pests. Drip irrigation pipes, 50 cm long, were installed between drippers to ensure even distribution of water. The irrigation system setup using waste water supplied by the organic fish farm. Plant growth was observed weekly during the whole plantation period to avoid any nutrient deficiencies. Chicken manure compost and organic fish farm water was sufficient enough to ensure proper plant growth. Hand picking of emerged weeds was carried out in the experimental greenhouse to prevent the competition between weeds and cultivated tomatoes.

Two different hybrid tomato varieties, Shifa and Savera, were bought from the Agriculture Research Center, Egypt (Shifa was imported from Thailand and Savera from China) and were sown on two different dates for the two different plantation periods (August 20 for the first plantation season and December 15, 2014 for the second plantation period in the horticulture nursery of the National Research Center). The tomato seeds were left for 30 days before transplanting under nursery conditions in order to obtain suitable germination and good plantlets before being transferred to the greenhouse. Tomato plantlets were transplanted on September 15, 2014 for the first plantation period and January 15, 2015 for the second plantation period. A strip plot design was used in the experimental greenhouse. It consisted of 3 replicates. Each replicate consisted of 6 double rows with a total of 12 rows for the block. The spaces between the double rows were 60–65 cm and the spaces between single rows ranged from 80 to 100 cm. The inter-row space was 50 cm as determined by the drippers in the pipeline. Spaces of 100–120 cm were left free between each replicate for experimental purposes. Each double row consisted of 40 transplants (20 plants per single row per block).

Field assay of Bemisia tabaci infestation suitability for the two tomato varieties

The purpose of this assay was to determine the difference in suitability of the studied tomato varieties for infestation by B. tabaci as a prophylactic measure. Sampling for B. tabaci was carried out after early detection using sticky traps (Yellow and Blue) for a period of 11 consecutive weeks, starting from early November 2014 to mid January 2015 for the first plantation period and from early March 2015 to mid May 2015 for the second plantation period, respectively. Twenty fully stretched new leaves for each variety were weekly inspected and observed in the greenhouse. The adults were counted for both tomato varieties in the greenhouse (Naranjo and Flint 1995).

Application of bio-rational insecticides

Four bio-rational insecticides were applied in this study namely, B. bassiana (Bio Power: Stanes Company), Metarhizium anisopliae (Bio Magic: Stanes Company), azadirachtin (Nimbecidine: Stanes Company), and spinosad (Tracer: Dow AgroSciences Company). The recommended dose given by the producer for field application was used and was assumed to be the median lethal concentration ($L_{50}$). The median lethal
concentrations of bio-rational insecticides are: 5 ml · l−1 · ha−1 for B. bassiana; 5 ml · l−1 · ha−1 for M. anisopliae; 5 ml · l−1 · ha−1 for azadirachtin and 0.3 ml · l−1 · ha−1 for spinosad. Additionally, higher and lower concentrations than the recommended doses (LC50) were used. The higher concentration was the lethal dose (LD) and was prepared by doubling the LC50. The lower concentration (SLD) was a double dilution to obtain the sub-lethal dose of LC25. The efficacy of each insecticide at the three aforementioned concentrations was evaluated in the greenhouse trials. The three replicates were each divided into two sub replicates. Each concentration was applied on two double rows out of a total of six rows. A plastic sheet was used to separate the different treatments during application. All the sprayings were done at 7 day intervals. The applications of insecticides were done by using 5 l knapsack sprayers for each bio-rational insecticide. Evaluation of the tested bio-rational insecticides against B. tabaci was performed through random leaf sampling. Twenty infested leaves from different concentrations were observed in the greenhouse 6 days after insecticide application for the presence of B. tabaci adults on both tomato varieties for a period of 6 consecutive weeks, starting from early December 2014 to mid-January 2015 for the first plantation period and from early April 2015 to mid-May 2015 for the second plantation period.

Statistical analysis

An independent t-test was carried out to find the significant differences between the Shifa and Savera F1 hybrid tomato varieties in the suitability of B. tabaci based on the number of adults. The SPSS program Version 16 (SPSS Inc. 2005) was used. The analysis of variance of the strip plot experiment for the results obtained from the efficacy of different bio-rational insecticides was carried out using the MSTAT-C Computer Software Program (MSTAT-C 1988). The least significant difference test was applied at 0.05 probability level to compare mean treatments.

Results and Discussion

Infestation suitability between tomato varieties under greenhouse conditions

Using a t-test a statistically significant difference in infestation suitability between two tomato F1 hybrid varieties, Shifa and Savera (2014–2015) under greenhouse conditions was found. Significantly high infestation was found in the Shifa F1 hybrid tomato variety compared to the Savera F1 hybrid tomato variety in the first season (Table 1). Significant differences were clearly seen during the 1st, 2nd, 3rd, 6th, 7th, 8th and 9th weeks. During the 4th, 5th, 10th and 11th weeks there were non-significant differences in infestation suitability between the two tomato varieties for the first plantation period. The greatest increase in infestation was recorded during the 7th and 8th weeks of the research and occurred during the 3rd and 4th weeks of December 2014 for both tomato varieties which had different levels of infestation (Fig. 1A). On the other hand, the lowest infestation was found in the two tomato varieties during the 1st, 2nd, 5th, 6th and 10th weeks of the

Table 1. Bemisia tabaci infestation suitability between Shifa and Savera tomato varieties under greenhouse conditions

<table>
<thead>
<tr>
<th>No. weeks</th>
<th>1st plantation period</th>
<th>2nd plantation period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shifa</td>
<td>Savera</td>
</tr>
<tr>
<td></td>
<td>no. adults (mean±SE)</td>
<td>no. adults (mean±SE)</td>
</tr>
<tr>
<td>1</td>
<td>3.80±0.25</td>
<td>2.00±0.19</td>
</tr>
<tr>
<td>2</td>
<td>4.15±0.44</td>
<td>2.00±0.23</td>
</tr>
<tr>
<td>3</td>
<td>5.30±0.32</td>
<td>3.75±0.23</td>
</tr>
<tr>
<td>4</td>
<td>5.10±0.50</td>
<td>4.00±0.51</td>
</tr>
<tr>
<td>5</td>
<td>4.00±0.37</td>
<td>3.50±0.29</td>
</tr>
<tr>
<td>6</td>
<td>4.60±0.27</td>
<td>3.25±0.32</td>
</tr>
<tr>
<td>7</td>
<td>6.00±0.41</td>
<td>3.85±0.26</td>
</tr>
<tr>
<td>8</td>
<td>6.20±0.46</td>
<td>4.00±0.38</td>
</tr>
<tr>
<td>9</td>
<td>5.80±0.38</td>
<td>3.55±0.28</td>
</tr>
<tr>
<td>10</td>
<td>5.10±0.31</td>
<td>4.20±0.33</td>
</tr>
<tr>
<td>11</td>
<td>3.55±0.34</td>
<td>2.55±0.27</td>
</tr>
</tbody>
</table>

n = 20; *, **, *** t is significant at the p < 0.05 level; ns – not significant
experiment for the first plantation period. During the second plantation period, there was a significant difference between the two tomato varieties; *B. tabaci* showed a preference for the Shifa F1 hybrid over the Savera F1 hybrid tomato variety. The differences were seen during the 1st, 2nd, 4th, 6th, 7th, 8th, and 10th weeks. There were no significant differences between the two tomato F1 hybrid varieties during the 3rd, 5th, 9th and 11th weeks, respectively (Table 1). According to Figure 1B, the greatest infestation was recorded from March 17 to April 23, 2015. The lowest infestation levels were recorded during the 1st and 2nd weeks of March and the 1st and 2nd weeks of May 2015.

The data agree with Arnó *et al.* (2005) and Wenh-Feng *et al.* (2009) who found that *B. tabaci* were more abundant in autumn than in spring and were very reduced during late winter. Moreover, Karut *et al.* (2012) reported that population densities of *B. tabaci* and infestation levels were higher in autumn than in spring (2008–2009 and 2009–2010). The differences in *B. tabaci* infestation levels between the Shifa F1 hybrid that have high infestation levels and the Savera F1 hybrid that have lower *B. tabaci* infestation levels could be due to the greater number of trichomes on the leaves and stems of the Savera variety than the number of trichomes on the leaves and stems of the Shifa variety. Cultivated tomato genotypes are covered with glandular and non-glandular types of trichomes on hypocotyls, stems, leaves, floral organs, and immature fruit. There is considerable diversity of trichome type, density and chemical composition within tomato species (Schilmiller *et al.* 2008). Numerous studies have shown that trichomes play an important role in the resistance of tomato species to insect infestation. Trichomes may contribute to resistance by impeding the ability of insects to feed, move, and survive on the plants (Kennedy 2003; Simmons and Gurr 2005). More importantly, resistance is also mediated by glandular trichome-borne metabolites that exert toxic effects on insect herbivores, or that physically entrap the insect upon rupture of the trichome gland. Differences in tomato infestation levels were reported by (JiRong and Ming 2011) because the hairy plant can repel aphids, *B. tabaci* and American leaf miners. Furthermore, Samarajeewa *et al.* (2005) found that the plants resistant to infestation with *B. tabaci* had more trichomes on their leaves and stems than the susceptible plants. Also, different compounds, such as acyl sugars, methyl ketones, terpenes, and alkaloids in tomato leaves, confer resistance to numerous insect pests of tomatoes, including aphids (*Macrosiphum euphorbiae* and *Myzus persicae*) and whitefly (*B. argentifolii*) (Rodriguez *et al.* 1993; Blauth *et al.* 1998).

**Efficacy of bio-rational insecticides**

In the present study, different bio-rational bio-pesticides were used to evaluate the efficacy of these bio-rational insecticides against *B. tabaci* adults under greenhouse conditions. The results from the 1st plantation period showed a significant difference between the treated and untreated (control) plants for Shifa and Savera F1 hybrid tomato varieties (Table 2 and Fig. 2A, B). These results could be easily calculated by the mean number of *B. tabaci* adults when treated with the different bio-rational insecticides for Shifa and Savera, respectively. With the different concentrations there were highly significant differences during the whole plantation period on Shifa and Savera F1 hybrid tomato varieties (Table 2 and Fig. 2A, B). These results could be easily calculated by the mean number of *B. tabaci* adults when treated with the different bio-rational insecticides for Shifa and Savera, respectively. With the different concentrations there were highly significant differences during the whole plantation period on Shifa and Savera F1 hybrid tomato varieties on the number of *B. tabaci* adults. The greater the concentration, the greater the efficacy on *B. tabaci* adult mortality gained. There were no significant differences between the LC$_{50}$ and the higher concentrations of the applied bio-rational insecticides (Table 3).

In the 2nd plantation period there were statistically significant differences between the treated and untreated plants for both Shifa and Savera F1 hybrid tomato varieties with $p = 0$, during the six weeks of applications. These significant differences can easily be observed in Figure 2C, D for Shifa and Savera F1
hybrid tomato varieties, respectively. Furthermore, there were significant differences between the different concentrations of each bio-rational insecticide when compared with each other as shown in Tables 2 and 4. In spite of the significant differences between the different bio-rational insecticides in comparison to untreated plants, spinosad and azadirachtin were the strongest in their efficacy on adult *B. tabaci* during the two plantation periods on both Shifa and Savera F1 hybrid tomato varieties. *Metarhizium anisopliae* and *B. bassiana* showed highly significant efficacy during the first plantation period while during the second plantation period they tended to have a moderate efficacy on *B. tabaci* adults.

According to the Abbott formula (1925), spinosad reduced the greatest percent of *B. tabaci* adults at LC50 (from 83.97 to 94.61%) for both Shifa and Savera F1 hybrid tomato varieties under greenhouse conditions for the two plantation periods (Fig. 3). The next most effective was azadirachtin which gave an approximate-est in their efficacy on adult *B. tabaci* during the two plantation periods. In the first plantation period *B. bassiana* and *M. anisopliae* achieved very satisfactory results on the *B. tabaci* adults. During the second plantation period *B. bassiana* and *M. anisopliae* caused almost 50 to 56.53% mortality of the *B. tabaci* adults.

Our results from the two plantation periods are similar to those of many authors who compared the efficacies of different microbial insecticides for integrated field management of the whitefly (Lacey et al. 2008; Lynn et al. 2010; Zhu and Kim 2011; Ghosh 2014; de Almeida Marques et al. 2014). Kim et al. (2011) studied the effect of different insecticides including spinosad on B and Q Bio-Types of *B. tabaci*. Their findings were comparable with what we found, showing the great efficacy of spinosad on *B. tabaci* populations. Moreover, Yin et al. (2011) investigated the efficacy of eight insecticides on *B. tabaci*. Their results showed that spinosad and Fipronil might be the best choices for the control of *B. tabaci* adults. Many researchers have investigated the efficacy of azadirachtin products against *B. tabaci* (Lynn et al. 2010; de Almeida Marques et al. 2014). De Almeida Marques et al. (2014) studied the effects of different plant extracts including neem for the management of *B. tabaci* Bio-Type B. A high efficacy of azadirachtin on *B. tabaci* infesting Solanaceae plants was found. However, Lynn et al. (2010) studied the effects of azadirachtin and neem based formulas on *B. tabaci*. They found a 78.2% reduction in the *B. tabaci* population which is in agreement with our results. Islam et al. (2011) also found the same significant effects of *B. bassiana* and azadirachtin on *B. tabaci*, namely, that there were no significant differences in mortality rates between the middle and sub-lethal doses used during their study.

Zhu and Kim (2011) reported different pathogenicity effects of different entomopathogenic fungi against *B. tabaci*. According to their results *B. bassiana* is very virulent and is considered to be one of the most virulent entomopathogenic fungi against *B. tabaci*. Lacey et al. (2008) studied the effect of the entomopathogenic fungi *B. bassiana* on the whitefly *B. tabaci*. They obtained
Fig. 2. Efficacy of the different bio-rational insecticides on *Bemisia tabaci* adults: 1st plantation period – Shifa (A) and Savera (B) variety, respectively; 2nd plantation period – Shifa (C) and Savera (D) variety, respectively.
Table 3. Efficacy of different bio-rational insecticides on *Bemisia tabaci* adults for the 1st plantation period

<table>
<thead>
<tr>
<th>Variety</th>
<th>Shifa</th>
<th>Saverana</th>
<th>Shifa</th>
<th>Saverana</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>week 1</td>
<td>week 2</td>
<td>week 3</td>
<td>week 4</td>
</tr>
<tr>
<td><strong>Beauveria bassiana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.80</td>
<td>4.50</td>
<td>5.50</td>
<td>5.85</td>
</tr>
<tr>
<td>SLD</td>
<td>5.20</td>
<td>2.95</td>
<td>3.00</td>
<td>3.55</td>
</tr>
<tr>
<td>MLD</td>
<td>0.75</td>
<td>0.60</td>
<td>0.95</td>
<td>1.20</td>
</tr>
<tr>
<td>LD</td>
<td>0.85</td>
<td>0.40</td>
<td>0.80</td>
<td>0.55</td>
</tr>
<tr>
<td><strong>F-value</strong></td>
<td>0.12 ns</td>
<td>0.22 ns</td>
<td>0.07 ns</td>
<td>0.07 ns</td>
</tr>
<tr>
<td><strong>LSD at 5%</strong></td>
<td>0.24</td>
<td>0.14</td>
<td>0.16</td>
<td>0.18</td>
</tr>
</tbody>
</table>

n = 20; *, **, *** is significant at the p < 0.05, 0.01, and 0.001 levels, respectively.

SLD – sub-lethal dose; MLD – median lethal dose; LD – lethal dose.

Table 4. Efficacy of different bio-rational insecticides on *Bemisia tabaci* adults for the 2nd plantation period

<table>
<thead>
<tr>
<th>Variety</th>
<th>Shifa</th>
<th>Saverana</th>
<th>Shifa</th>
<th>Saverana</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>week 1</td>
<td>week 2</td>
<td>week 3</td>
<td>week 4</td>
</tr>
<tr>
<td><strong>Beauveria bassiana</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.80</td>
<td>7.35</td>
<td>7.95</td>
<td>7.90</td>
</tr>
<tr>
<td>SLD</td>
<td>4.60</td>
<td>5.50</td>
<td>3.90</td>
<td>3.65</td>
</tr>
<tr>
<td>MLD</td>
<td>3.80</td>
<td>4.20</td>
<td>2.95</td>
<td>2.75</td>
</tr>
<tr>
<td>LD</td>
<td>3.65</td>
<td>3.90</td>
<td>3.25</td>
<td>2.40</td>
</tr>
<tr>
<td><strong>F-value</strong></td>
<td>0***</td>
<td>0***</td>
<td>0***</td>
<td>0***</td>
</tr>
<tr>
<td><strong>LSD at 5%</strong></td>
<td>0.24</td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
</tr>
</tbody>
</table>

n = 20; *, **, *** is significant at the p < 0.05, 0.01, and 0.001 levels, respectively.

SLD – sub-lethal dose; MLD – median lethal dose; LD – lethal dose.
a reduction in the *B. tabaci* population of about 41 to 50% when compared with the untreated plants. Similar findings were obtained by Liu et al. (1999) and Ota et al. (1999) and were comparable to our results. Lacey et al. (2008) found that *M. anisopliae* reduced the *B. tabaci* population on eggplant by 30 to 90%. Batta (2003) obtained exactly the same results as we did. Santiago-Alvarez et al. (2006) also discovered the efficacy of *B. bassiana* on tomato and other host plants of *B. tabaci* to cause about 68 to 73% mortality of *B. tabaci*.

In conclusion, there were significant differences in infestation suitability between the studied varieties (Shifa and Savera) during the plantation periods. Significantly higher infestation was found in the Shifa F1 hybrid tomato variety than in the Savera F1 hybrid tomato variety. Therefore we recommend planting the Savera F1 hybrid tomato variety under greenhouse conditions during the tested periods. Concerning the utilization of bio-rational insecticides – previous research has indicated that when there are no control measures applied to tomato plants, there will be a 100% reduction in the produce. In our experimental trial for the evaluation of different bio-rational insecticides we found that spinosad, azadirachtin, *B. bassiana* and *M. anisopliae* had varied effects against *B. tabaci* on greenhouse tomatoes. The reduction in *B. tabaci* infestation under all the applications varied between 50 and 94.61% for the two plantation periods. Spinosad reduced the *B. tabaci* population by 83 to 94.61% and is ranked as the most effective bio-rational insecticide among the tested insecticides. It is followed by azadirachtin which reduced the *B. tabaci* population by 74 to 86.11%. *Metarhizium anisopliae* and *B. bassiana* gave a percent reduction of 52 to 89% and 50 to 87%, respectively. These different levels of efficacies on the target insect pest were translated into a 30–50% increase in total tomato produce when compared to the total harvested plants. This obvious increase merits paying attention to the cost benefits of the different bio-rational insecticides.

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