Comparison of the toxicity of three botanical extracts on the second nymph of the citrus mealybug Planococcus citri (Risso) under nursery and laboratory conditions

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Abstract

Botanical insecticides play an important role in sustainable agriculture. The effect of Tondexir (pepper extract), Sirinol (garlic extract) and Palizin (eucalyptus extract) on Citrus Mealybug (CM) Planococcus citri (Risso) (Homoptera: Pseudococcidae) and Coccinellid Predator (CP) Cryptolemus montrazieri was investigated. Citrus Mealybug was reared on Butternut pumpkin (2-3 kg). Factorial experimental design with random blocks with six replicates was used for each botanical insecticide under nursery conditions. Each toxin was tested separately and CM mortality was recorded at 24, 48, 72 and 96 hours after treatment. Tondexir (3000 ppm) resulted in the highest CM mortality (90.96±2.93%), followed by Palizin (3000 ppm) (89.16±1.92%) and Sirinol (3500 ppm) (87.11±1.11%) 96 h after treatment. Adult emergence was prevented by Tondexir (3000 ppm), Palizin (3000 ppm) and Sirinol (3500 ppm). Among three botanical insecticides, the least LC50 was obtained by Palizin with 811.297 ppm. There were no toxic effects due to the use of the above mentioned botanical pesticides on any of the Coccinellid Predator (CP) Cryptolemus montrazieri Multisant (Coleoptera: Coccinellidae) stages.

Keywords: Tondexir, Palizin, Sirinol, Citrus Mealybug (CM), Planococcus citri, Coccinellid Predator (CP), Cryptolemus montrazieri.

Introduction
Citrus mealybug (CM), Planococcus citri, is one of the most important pests of citrus in the world. Keriokhin was the first person who identified this pest in Iran (16). Most of the research regarding the effect of pesticides on CM has been carried out in South Africa, Palestine, Egypt, India, America, Spain, USA and Japan (19). The use of synthetic insecticides has led to numerous problems unforeseen at the time of their introduction, in addition to the evolution of pesticides resistance in pest populations (10). The Coccinellid predator (CP) is one of the biological control agents for the control of CM and is inhibited by chemical pesticides (14). Many different pesticides such as Profenofos, Methidathion, Chlorpyrifos, fenpropatrin and Methomyl have been used to control CM (2) but are found harmful to coccinellid beetles (18).

The above mentioned negative effects of using conventional insecticides generated a need to find alternative ways of controlling target pest species (23). Botanical insecticides can be an alternative to conventional pesticides as they are effective against pests, remain for a short period in the field, and are safe and cheap. Four Biological pesticides, Biofly (Beauveria bassiana), Biovar (Beauveria bassiana), Bioranza (Metarhizium anisopliae) and orange oil plus two chemical pesticides, Admiral (pyriproxyfen) and Cidential (phenthoate), were used against the pink hibiscus mealybug Macconellicoccus hirsutus (Green) (Homoptera: Pseudococcidae). The highest mortality was obtained from Cidential treatment (3). Amongst various options, aqueous extract of neem seeds (kernels of Azadirachta indica), Allium sativum, Lantana camara, Annona squamosa, carica papaya, etc. were highly effective for the control of coffee mealy bugs (21).

The aim of this study was to investigate the effect of Tondexir (pepper extract), Sirinol (garlic extract) and Palizin (Eucalyptus extract) on Citrus Mealybug, Planococcus citri (Risso) (Homoptera: Pseudococcidae) in semi-field conditions.

Materials and Methods
The botanical insecticides used are listed in Table 1. This research was done in semi-field condition in Randomized Complete Block Design in three replicate at Sari Agricultural University.

Rearing insects (Planococcus citri):
Semi field assay and experimental design: The toxicity of the three different botanical insecticides (Table 1) to CM was examined in the nursery at Sari Agricultural Sciences and Natural Resources University in 2010 & 2011. Seventy-two young trees (4 years) of the citrus variety Thomson Navel (Citrus sinensis) nursery within Sari Agricultural University and spray bioassay method were used. Experiments for each treatment were replicated four times, and distilled water was used as a control. At 24, 48, 72 and 96 hours post-treatment, the numbers of live and dead second instar nymphs in each replicate were counted in the laboratory under a stereomicroscope.

The rearing protocol followed was as described by Gerald-Robison (12) and Gharizadeh (13) with some modifications. Citrus Mealybug was reared on Butternut pumpkin Cucurbita moschata (2-3 kg) in plastic containers (25 cm diameter x 4 cm high) at 25±3°C and 16:8 hrs (dark:light) photoperiod in an incubator. The tops of the containers were covered by Vaseline oil to prevent CM escape and then these CM were used to infest young trees in the nursery.
Table 1. Botanical insecticides used in this research.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Trade name</th>
<th>Chemical group</th>
<th>Formulation</th>
<th>Dose (ml/1000 Lit water)</th>
<th>LD₅₀ (mg/kg)</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insecticidal gel (IG)</td>
<td>Sirinol</td>
<td>Garlic Extract in edible oil</td>
<td>EC</td>
<td>0-3500/1000</td>
<td>&gt; 5000</td>
<td>Kimia sabzavar Co.</td>
</tr>
<tr>
<td>Insecticidal soap (IS)</td>
<td>Palizin</td>
<td>Coconut soap 65%</td>
<td>SL</td>
<td>0-3000/1000</td>
<td>&gt; 5000</td>
<td>Kimia sabzavar Co.</td>
</tr>
<tr>
<td>Insecticidal emulsion (IE)</td>
<td>Tondexir</td>
<td>Hot red pepper extract in edible oil</td>
<td>EC</td>
<td>0-3000/1000</td>
<td>&gt; 5000</td>
<td>Kimia sabzavar Co.</td>
</tr>
<tr>
<td>Fenpropathrin</td>
<td>Danitol</td>
<td>Pyrethroid</td>
<td>SL</td>
<td>0-3000/1000</td>
<td>&gt; 5000</td>
<td>Bayer</td>
</tr>
</tbody>
</table>

Insecticides application
Five doses for each insecticide were chosen based on pilot experiments, plus a control.

Tondexir and Palizin were used with 0, 500, 1000, 1500, 2000 and 3000 ppm concentration, and Sirinol with 0, 1000, 1500, 2000, 2500 and 3500 ppm concentration. Insect mortality was recorded 24, 48, 72 and 96 hours after treatment.

Statistical analysis
The percentage of mortality was recorded from each treatment and any mortality in the control was corrected using Abbott’s formula (1). Analysis of variance (ANOVA) was done using the spss program and the comparison of the means was done by Tukeys test (8). The LC₅₀ and LC₉₀ were calculated by polo+ program version 1 2002-2012 Leora software⁶.

Results
The analysis of variance (ANOVA) has shown that there were significant differences among different concentrations (factor A) and also among different times (factor B) and there were no significant differences for the interaction between dose and time (Table 2).

The results have also shown that the highest mortality of CM was obtained when using the 3000 ppm dose of Palizin and Tondexir (89.16±1.92% and 90.96±2.93% respective mortality) and at the 3500 ppm dose of Sirinol (87.11%±1.11% mortality). The lowest mortality was obtained with Palizin and Tondexir at the 500 ppm dose and by Sirinol at the 1000 ppm dose. There were significant differences among different doses of the above three toxins on P. citri (Table 3).

The highest mortality was obtained at 72 hrs and 96 hrs post-treatment, and there was no significant difference between 72 hrs and 96 hrs post-treatment when using Palizin and Sirinol. The highest mortality obtained with Tondexir was at 96 hrs post-treatment. The highest mortality for each of the toxins was obtained 96 hrs post-treatment (Table 4).

The effect of different concentrations of botanical insecticides on P. citri at different times post treatment is shown in Table 5. There were significant differences between different doses of all toxins and between the toxins and the control. The highest mortality was obtained at the 3000 ppm dose of Palizin and Tondexir, and at the 3500 ppm dose of Sirinol, 96 hrs post-treatment (Table 5).

Table 2. The analysis of variation (ANOVA) of the effect of three botanical insecticides on mortality rate of the second nymphs of Planococcus citri.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Palizin</th>
<th>Sirinol</th>
<th>Tondexir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>58.55</td>
<td>05.315</td>
<td>09.105</td>
</tr>
<tr>
<td>Doses</td>
<td>5.10807</td>
<td>33.9616</td>
<td>18.11225</td>
</tr>
<tr>
<td>Times</td>
<td>52.988</td>
<td>11.1319</td>
<td>59.846</td>
</tr>
<tr>
<td>Doses x Times</td>
<td>20.80</td>
<td>56.80</td>
<td>106.80</td>
</tr>
<tr>
<td>Error</td>
<td>13.47</td>
<td>16.52</td>
<td>07.70</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>53.15</td>
<td>99.15</td>
<td>93.17</td>
</tr>
</tbody>
</table>

** Significant differences at P=0.01
Table 3. Effect of different concentrations of three botanical insecticides on mean mortality rate of *Planococcus citri*.

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>Palizin</th>
<th>Tondexir</th>
<th>Dose (ppm)</th>
<th>Sirinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>24.73 e</td>
<td>27.91 e</td>
<td>0.00</td>
<td>35.13 d</td>
</tr>
<tr>
<td>500</td>
<td>38.27 d</td>
<td>41.66 d</td>
<td>1000</td>
<td>40.41 d</td>
</tr>
<tr>
<td>1000</td>
<td>52.63 c</td>
<td>53.74 c</td>
<td>1500</td>
<td>45.00 c</td>
</tr>
<tr>
<td>1500</td>
<td>58.47 b</td>
<td>64.13 b</td>
<td>2000</td>
<td>61.11 b</td>
</tr>
<tr>
<td>2000</td>
<td>89.16 a</td>
<td>90.96 a</td>
<td>2500</td>
<td>87.11 a</td>
</tr>
<tr>
<td>3000</td>
<td>1.80 f</td>
<td>1.87 f</td>
<td>3500</td>
<td>2.03 e</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different at P=0.01.

Table 4. *Planococcus citri* mortality rate as influenced by different doses of toxins observed at different periods after treatment.

<table>
<thead>
<tr>
<th>Hours post treatment</th>
<th>Palizin</th>
<th>Sirinol</th>
<th>Tondexir</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>45.34 c</td>
<td>59.34 c</td>
<td>47.37 c</td>
</tr>
<tr>
<td>48</td>
<td>84.42 b</td>
<td>19.42 b</td>
<td>72.45 b</td>
</tr>
<tr>
<td>72</td>
<td>86.47 a</td>
<td>87.49 a</td>
<td>25.50 ab</td>
</tr>
<tr>
<td>96</td>
<td>59.51 a</td>
<td>94.53 a</td>
<td>21.53 a</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different at P=0.01.

Table 5. *Planococcus citri* mortality rate (%) means as influenced by different doses of toxins from botanical extracts observed at different times after treatment.

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>Hours after treatment</th>
<th>Tondexir</th>
<th>Palizin</th>
<th>Dose (ppm)</th>
<th>Hours after treatment</th>
<th>Sirinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>24</td>
<td>11.66 gh</td>
<td>10.55 fg</td>
<td>0.0</td>
<td>24</td>
<td>20.00 fg</td>
</tr>
<tr>
<td>0.0</td>
<td>48</td>
<td>27.78 fg</td>
<td>21.11 eg</td>
<td>0.0</td>
<td>48</td>
<td>27.22 fg</td>
</tr>
<tr>
<td>0.0</td>
<td>72</td>
<td>32.22 eg</td>
<td>30.55 dg</td>
<td>0.0</td>
<td>72</td>
<td>43.89 cf</td>
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<tr>
<td>0.0</td>
<td>96</td>
<td>40.00 df</td>
<td>36.66 cg</td>
<td>0.0</td>
<td>96</td>
<td>49.44 bf</td>
</tr>
<tr>
<td>500</td>
<td>24</td>
<td>30.22 fg</td>
<td>31.55 dg</td>
<td>1000</td>
<td>24</td>
<td>28.89 eg</td>
</tr>
<tr>
<td>500</td>
<td>48</td>
<td>38.22 df</td>
<td>35.77 dg</td>
<td>1000</td>
<td>48</td>
<td>38.33 dg</td>
</tr>
<tr>
<td>500</td>
<td>72</td>
<td>45.77 df</td>
<td>40.88 bf</td>
<td>1000</td>
<td>72</td>
<td>44.44 cf</td>
</tr>
<tr>
<td>500</td>
<td>96</td>
<td>53.44 be</td>
<td>44.89 bf</td>
<td>1000</td>
<td>96</td>
<td>50.00 bf</td>
</tr>
<tr>
<td>1000</td>
<td>24</td>
<td>46.66 de</td>
<td>43.73 bf</td>
<td>1500</td>
<td>24</td>
<td>30.55 fg</td>
</tr>
<tr>
<td>1000</td>
<td>48</td>
<td>52.77 be</td>
<td>51.11 be</td>
<td>1500</td>
<td>48</td>
<td>44.44 cf</td>
</tr>
<tr>
<td>1000</td>
<td>72</td>
<td>57.77 bd</td>
<td>57.77 ae</td>
<td>1500</td>
<td>72</td>
<td>48.33 bf</td>
</tr>
<tr>
<td>1000</td>
<td>96</td>
<td>57.77 bd</td>
<td>58.33 ae</td>
<td>1500</td>
<td>96</td>
<td>56.66 af</td>
</tr>
<tr>
<td>1500</td>
<td>24</td>
<td>48.38 ef</td>
<td>42.77 bf</td>
<td>2000</td>
<td>24</td>
<td>47.22 bf</td>
</tr>
<tr>
<td>1500</td>
<td>48</td>
<td>61.55 bd</td>
<td>53.59 be</td>
<td>2000</td>
<td>48</td>
<td>55.55 af</td>
</tr>
<tr>
<td>1500</td>
<td>72</td>
<td>71.66 ac</td>
<td>62.77 ad</td>
<td>2000</td>
<td>72</td>
<td>68.33 ae</td>
</tr>
<tr>
<td>1500</td>
<td>96</td>
<td>75.00 ac</td>
<td>74.44 ac</td>
<td>2000</td>
<td>96</td>
<td>73.33 ad</td>
</tr>
<tr>
<td>2000</td>
<td>24</td>
<td>86.11 a</td>
<td>76.66 ab</td>
<td>2500</td>
<td>24</td>
<td>78.89 ac</td>
</tr>
<tr>
<td>2000</td>
<td>48</td>
<td>92.22 a</td>
<td>93.33 a</td>
<td>2500</td>
<td>48</td>
<td>85.55 ab</td>
</tr>
<tr>
<td>2000</td>
<td>72</td>
<td>92.22 a</td>
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<td>2500</td>
<td>72</td>
<td>92.22 a</td>
</tr>
<tr>
<td>2000</td>
<td>96</td>
<td>92.20 a</td>
<td>93.33 a</td>
<td>2500</td>
<td>96</td>
<td>92.22 a</td>
</tr>
<tr>
<td>3000</td>
<td>24</td>
<td>1.87 h</td>
<td>1.87 g</td>
<td>3500</td>
<td>24</td>
<td>2.03 g</td>
</tr>
<tr>
<td>3000</td>
<td>48</td>
<td>1.87 h</td>
<td>1.87 g</td>
<td>3500</td>
<td>48</td>
<td>2.03 g</td>
</tr>
<tr>
<td>3000</td>
<td>72</td>
<td>1.87 h</td>
<td>1.87 g</td>
<td>3500</td>
<td>72</td>
<td>2.03 g</td>
</tr>
<tr>
<td>3000</td>
<td>96</td>
<td>1.87 h</td>
<td>1.87 g</td>
<td>3500</td>
<td>96</td>
<td>2.03 g</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different at P=0.01.
The LC$_{50}$ of Palizin was 811.297 ppm, followed by Tondexir (821.716 ppm) and Sirinol (1562.125 ppm) (Table 6). There was no toxicity effect of Tondexir and Palizin and Sirinol on all stages of the Coccinellid Predator. 96 hrs post-treatment.

Table 6. The LC$_{50}$ and LC$_{90}$ of different toxins on the second instar nymphs of Planococcus citri.

<table>
<thead>
<tr>
<th>LC (ppm)</th>
<th>Tondexir</th>
<th>Palizin</th>
<th>Sirinol</th>
<th>Phenpropathrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC$_{50}$</td>
<td>821.7</td>
<td>811.3</td>
<td>1562.1</td>
<td>414.9</td>
</tr>
<tr>
<td>LC$_{90}$</td>
<td>3452.9</td>
<td>3672.6</td>
<td>4937.7</td>
<td>1083.3</td>
</tr>
</tbody>
</table>

Discussion

This study is the first to quantitatively demonstrate that commercially available plant extract products vary in their efficacy against certain arthropod pests. The popularity of “organic” products and the general interest in sustainable crop production led to an extended interest in using “natural” products, such as plant extracts, for pest control.

The effect of these three botanical insecticides as a biological control of P. citri has been evaluated here for the first time. Many chemical insecticides have been used to control P. citri; methomyl, dimethoate and buprofezin the Insect Growth Regulators (IGR), which decreased the insect population, but they are harmful to natural enemies (9, 22).

This study showed that botanical insecticides are preferable for the control of this insect pest due to having fewer negative effects on natural enemies of the target pests than conventional chemical insecticides. The effect of botanical extracts on mealy bugs is slower than synthetic insecticides, but they are eco-friendly and comparatively non-toxic or less toxic to human beings and easily degradable (3, 21).

Tondexir, Sirinol and Palizin were effective for controlling P. citri, with Tondexir producing the highest mortality of citrus mealybug, at 3000 ppm. This result was similar to what was obtained by Mohamed et al. (20). The high mortality obtained by using Tondexir and Palizin at 3000 ppm, and with Sirinol at 3500 ppm were in agreement with the result obtained earlier by Hollingsworth (15). These pesticides had no toxic effects on the coccinellids which is in agreement to what was obtained by Aida et al. (3). The same results were obtained earlier when using Palizin and Tondexir and Bacillus thurigiensis plus mineral oils against citrus leafminer (4, 5, 6, 7). Tondexir, Palizin and Sirinol are promising botanical insecticides and have the potential to be a significant component of an IPM program.

Several pest management tools must often be used simultaneously to deal with pest insect species. Natural plant extracts and biological control agents are two such tools that are used simultaneously because of their effectiveness, potential compatibility, and minimal risk to the environment and human health. Results of this study indicated that botanical pesticides conserve coccinellid predators better than the chemical pesticide fenpropathrin. Hence, it can be concluded that botanical pesticides were the most favorable insecticides for use in controlling P. citri because they kill the target pest but have no effect on natural enemies.

However, chemical toxins produced by plants may not only be detrimental to insect and mite pests (e. g. Palizin, Tondexir and Sirinol), but also to humans (11). For example, peppermint and rosemary oil have been shown to be effective against head louse, Pediculus humanus capitis De Geer (24) but contact dermatitis and occupational asthma have been attributed to exposure to rosemary oil (17).

The results of this study demonstrated that only a few selected plant-derived essential products available to consumers were effective in controlling specific arthropod pests. There was considerable variation in citrus mealybug mortality in response to the plant-derived essential oil products evaluated, which included products containing the same active ingredients. For example, the use of two canola oil products Garden Safe Houseplant and Garden Insect Spray (ready to use formulation) and Pyola, a canola oil concentrate, led to citrus mealybug mortality values of 74% and 50%, respectively.

Further research is needed to establish cultivation guidelines to ensure consistent quality of plant extract products. It is also important to assess phytotoxicity amongst commonly grown ornamental plants before introducing plant extract products to the marketplace and field testing for several years is needed.

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3. Aida, H.M., F.M. Saber, H.A. Ahmed and A.A. Sayed. 2010. Efficiency of certain insecticides on the population(s) of the pink hibiscus mealybug Maconellicoccus hirsutus (Green) and their natural enemies under the field condition in Ismailia governorate, Egypt. Academic Journal of Biological Sciences, 2: 11-17


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