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The toxicity and synergistic effects of alkyl-succinate oil on the first nymphal instars of the citrus cottony scale, *Pulvinaria aurantii* Cock. (Hem.: Coccidae)

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The citrus cottony scale, *Pulvinaria aurantii* Cock. (Hem.: Coccidae) is one of the most important citrus pests in Iran. This study investigated the toxicity and synergistic effects of organophosphorous insecticides (Malathion, Chlorpyrifos, Buprofezin and DG oil) on first nymphal instars of *P. aurantii* in the laboratory conditions (25 ± 1 °C, 75 ± 5% RH and 16L:8D photoperiod). The values of LC\(_{50}\) for Malathion, Chlorpyrifos and Buprofezin insecticides on the first nymphal instars were calculated as 76.90, 105.25 and 24.70 ppm, respectively. Also, results indicated that Buprofezin and Pyriproxifen are the most effective insecticides on first nymphal instars of *P. aurantii*. The values of LC\(_{50}\) for each insecticide without oil and in half dose along with DG oil were equal. This was an indication for synergism role of DG oil combination. The results of this study showed that DG oil not only decreases the consumption of insecticides, but also increases their efficacy.

**Keywords:** citrus cottony scale; DG oil; malathion; chlorpyrifos; buprofezin

**Introduction**

Citrus is one of the fruits in tropical places of the world that has an important role in human health. Also, it has a remarkable economical role in countries like the USA, Brazil, Spain, Japan, Iran, etc. Citrus cottony scale, *Pulvinaria aurantii* Cock. (Hem.: Coccidae), entered in Iran during 1937–1939 and was first seen in Rasht and Bandar-anzali citrus gardens of Iran (Behdad 1991). Now, this pest is spread throughout north of Iran from Bandar-anzali to Gorgan (Damavandian 2009). This pest attacked all types of citrus such as orange, sour orange, tangerine and so on, but its most suitable host is sour orange (Esmaeili 1991). Soft scales are one of the most important pests in crops, orchards and ornamental plants. Determining their exact economical importance is difficult due to feeding from molasses of plants, but in outbreak conditions, they caused leaf and fruit loss and the host plant to be dried. Soft-scale insects of all ages feed by sucking sap. They are found on all parts of the plant but are most noticeable on the fruit. Heavy infestations may cause discoloration, shoot distortion and leaf drop. The fruit may become pitted and

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unmarketable. The secretion of honeydew is also produced allowing for sooty mould development and decreases photosynthesis level. Moreover, it decreases the beauty of plants, fruits, and their market value with marking a dirty and black surface (Williams & Kosztarab 1972). Damage intensity of the citrus cottony scale, *P. aurantii*, during recent years causes insecticides to be used in large parts of citrus gardens in Mazandaran province of Iran for controlling the pest. Increasing the resistance of pests to insecticides is a difficult and critical matter in the world (Roush & Tabashnik 1990). The combination of several insecticides was used for controlling the pests. So, interaction between insecticides when they are used may include additive, synergism and antagonism effects (Duffus & Worth 1996). Damavandian (2003), examined LC$_{50}$ effect of Volk oil on first and second nymphal instars and adult females of the citrus cottony scale. Damavandian (2008) compared the common insecticides and mineral oils on controlling *P. aurantii* in citrus gardens and their effects on the predatory phytoseiid mites’ family. They had pointed to the role of oil in decreasing the mortality of predatory mites. Gholamian et al. (2010) investigated the effects of emulsifiable oil to control the citrus cottony scale. They concluded that three-time oil spray (in winter 1.5% concentration and fallowing spray in early June and September with 0.5% concentration) and also two-time oil spray (early June and September with 0.5% concentration) have the most effect on controlling *P. aurantii*. The most important synergistic advantage of insecticides is the control of pest resistance. This study was considered in order to examine the synergistic role of DG oil in combination with different insecticides on the cottony citrus scale, *P. aurantii*.

**Materials and methods**

**Collecting and rearing of *P. aurantii***

According to the studies, sour orange plant is the most susceptible host for rearing of the citrus cottony scale (Hallaji-sani 1999). The used plants were two-year old, and were grown in Iran Citrus Research Institute, Ramsar. Infested sour orange plants were placed in germinator with 25 ± 1 °C, 75 ± 5% RH and 16L:8D photoperiod.

**Bioassay experiments**

The used insecticides were Malathion, Chlorpyrifos and Buprofezin along with DG oil. For doing bioassay experiments, first nymphal instars which were immobile were used. Thus, contaminated leaves were separated from the plants and 10 first nymphal instars on each leaf were remained and others were omitted. In the first step during preliminary experiments, the concentrations with about 25 to 75% mortality level were used for final experiments. For each experiment, stock solution with high concentration was made, and the other concentrations were made using this equation: $N_1 \times V_1 = N_2 \times V_2$. In this equation, $N_1$ and $N_2$ are concentrated and more diluted doses and $V_1$ and $V_2$ are their volumes. In this experiment, for each insecticide, five concentrations were prepared and from each dose, 100 ml was used. To avoid precipitate solutions, before dipping the leaves, they were homogenised with shaker. For better sticking of insecticides to leaves, emulsifier of Tween 80 with the amount of 0.02% was used. For each concentration, 30 nymphs were used. Then, the end of leaves was covered by cottons and was placed in disposable containers.
Samples were kept in germinator under 25 ± 1 °C, 75 ± 5% RH and 16L:8D photoperiod. Nymphal mortality was calculated after 24 h. In order to determine the toxicity of DG oil, bioassay experiments, like other treatments, were done. It differs only in the mortality rate of nymphs that was counted after 48 h. In the experiment, Buprofezin insecticide was used with Sc40% formulation. However, since the Buprofezin is related to Insect Growth Regulator insecticides, the mortality level of nymphs was counted after about four days. After calculating the LC50 of DG oil, the oil was combined with half dose of the aforementioned insecticides, and in this form, the LC50 value and mortality rate were calculated.

**Data analysis**

The collected data was analysed with SAS software (SAS 2004) and LC50 for different insecticides, and the application of DG oil along with these insecticides were calculated.

**Results**

*Bioassay of different insecticides on the first nymphal instars*

The calculated 50% lethal concentration (LC50) values for different insecticides on the first nymphal instars of the citrus cottony scale, *P. aurantii*, are shown in Table 1. LC50 values for Chlorpyrifos, Malathion, Buprofezin and DG oil on first nymphal instars of *P. aurantii* was calculated 105.76, 251.9, 24.70 and 1143 ppm, respectively. The results showed that all the examined insecticides caused mortality in first nymphal instars of pest. Considering the upper and lower limits of LC50 value, different insecticides were not overlapped with each other, so their toxicity was differed.

Overlapping method and ratio proportion were used to compare different insecticides. In this method, LC50 of all insecticides was divided by the lowest LC50. It can be understood from Table 2 that Chlorpyrifos insecticide has the lowest ratio proportion, and Malathion and DG oil are the next ones.

Another method of comparison is using the overlapping graph. In this method, LC50 and its lower and higher limits were used. As it is shown in Figure 1, Malathion and Chlorpyrifos insecticides overlap with each other. It means that these two insecticides do not have a significant difference and had similar toxicity on the *P. aurantii* nymphs, but they have significant difference with Buprofezin and DG oil.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>N</th>
<th>LC50 (ppm)</th>
<th>95% Confidence limit (upper–lower)</th>
<th>Slope ± SE</th>
<th>Intercept ± SE</th>
<th>χ²</th>
<th>Pr &gt; chisq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>180</td>
<td>105.76</td>
<td>73.43–153.91</td>
<td>1.51 ± 0.27</td>
<td>−3.07 ± 0.5</td>
<td>0.83</td>
<td>0.84</td>
</tr>
<tr>
<td>Malathion</td>
<td>180</td>
<td>251.9</td>
<td>131.25–467.35</td>
<td>0.7 ± 0.12</td>
<td>−1.9 ± 0.33</td>
<td>2.08</td>
<td>0.71</td>
</tr>
<tr>
<td>Buprofezin</td>
<td>150</td>
<td>24.70</td>
<td>13.96–39.77</td>
<td>1.13 ± 0.18</td>
<td>−1.85 ± 0.32</td>
<td>1.61</td>
<td>0.65</td>
</tr>
<tr>
<td>DG</td>
<td>150</td>
<td>1143</td>
<td>536.34–2640</td>
<td>0.65 ± 0.11</td>
<td>−2 ± 0.34</td>
<td>2.51</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Bioassay of first nymphal instars of the citrus cottony scale, *P. aurantii*.

Table 2. Comparison of ratio proportion of 50% lethal concentration (LC₅₀) of different insecticides without oil on the citrus cottony scale, *P. aurantii*.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyritos + DG</td>
<td>4.28</td>
</tr>
<tr>
<td>Buprofezin + DG</td>
<td>10.19</td>
</tr>
<tr>
<td>Malathion + DG</td>
<td>46.27</td>
</tr>
</tbody>
</table>

Table 3. Bioassay of first nymphal instars of the citrus cottony scale, *P. aurantii*, by different insecticides along with DG oil.

<table>
<thead>
<tr>
<th>Insecticide + DG oil</th>
<th>N</th>
<th>LC₅₀ (ppm)</th>
<th>95% Confidence limit (upper–lower)</th>
<th>Slope ± SE</th>
<th>Intercept</th>
<th>$\chi^2$</th>
<th>Pr &gt; chisq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyritos + DG</td>
<td>180</td>
<td>298.12</td>
<td>163.88–542.04</td>
<td>0.87 ± 0.16</td>
<td>−2.17 ± 0.41</td>
<td>1.68</td>
<td>0.79</td>
</tr>
<tr>
<td>Malathion + DG</td>
<td>180</td>
<td>156.60</td>
<td>85.27–296.90</td>
<td>0.81 ± 0.14</td>
<td>−1.78 ± 0.32</td>
<td>1.26</td>
<td>0.86</td>
</tr>
<tr>
<td>Buprofezin + DG</td>
<td>150</td>
<td>26.19</td>
<td>13.62–44.07</td>
<td>1.10 ± 0.17</td>
<td>1.43 ± 0.31</td>
<td>1.63</td>
<td>0.65</td>
</tr>
</tbody>
</table>

**Bioassay of different insecticides along with oil on first nymphal instars**

The calculated 50% lethal concentration (LC₅₀) values for different insecticides along with DG oil on the first nymphal instars of the citrus cottony scale are shown in Table 3. LC₅₀ values for Chlorpyritos, Malathion, and Buprofezin plus DG oil on first nymphal instars of *P. aurantii* were calculated 298.12, 156.60 and 26.19 ppm, respectively.
Comparison of LC50 values indicated that DG oil along with Buprofezin has reduced LC50 to the lowest level. It means that it is the most toxic combination of insecticide and DG oil on the P. aurantii nymphs.

As it can be understood from Table 4, the ratio proportion of Chlorpyrifos + DG to Buprofezin + DG is bigger. This means that combination of Chlorpyrifos with DG is less toxic than the combination of Malathion and Buprofezin with DG.

Also, in overlapping graph (Figure 2), the combination of Chlorpyrifos and Malathion with DG oil overlapped with each other. They were overlapped with the combination of Buprofezin with DG. It means that the combination of Chlorpyrifos and Malathion insecticides with DG oil did not show a significant difference, and they have similar effect on the pest. But, they had significant difference with the combination of Buprofezin with DG oil.

**Discussion**

Results of this study showed that combination of Buprofezine with DG oil had the lowest LC50 value, i.e. it had the highest toxicity. It also showed that all the insecticides along with DG oil had the similar LC50, although they were used in half dose of the recommended concentration. That is, the combination of DG oil had an important role in decreasing insecticides consumption. According to Vojoudi et al. (2011), LC50 value of Chlorpyrifos insecticide on third larval instars of Helicoverpa armigera was
calculated 4.6 ppm and this value is less than the results of this study. Probably, this difference is due to susceptibility of insect species. Hosseininoveh et al. (2009) examined the synergistic effects of Citoweet oil in combine with Pirimicarb on the greenhouse whitefly. And the calculated LC$_{50}$ value of Citoweet oil and Pirimicarb was 0.98 and 0.26, respectively. LC$_{25}$ values of these two with each other were 0.043 and 0.25, respectively. Damavandian (2009) examined the LC$_{50}$ level of Volk mineral oil on the first, second nymphal instars and adult females and its calculated value on the first and second nymphal instars was 0.593 and on female was 1.61 ppm.

According to Kljajic et al. (2006), LD$_{50}$ values of Malathion on the sawtoothed grain beetle adult insects of *Sitophilus granarius*, *S. oryzae*, *S. zeamais* after 24 h were calculated as 8.32, 16.64 and 10.62 mg/cm$^2$.

Montazeri and Alavi (2001) were carried out some experiments on *P. aurantii* to examine the effect of emulsifiable oil alone and in combine with different concentrations of Azinphos-methyl insecticide. Their results showed that the lowest dose (75% oil alone) is recommended. Jafari (2005) evaluated the effects of new insecticide, Buprofezin, against the citrus cottony scale in comparison with common insecticides in Mazandaran gardens of Iran. His results indicated that Buprofezin does not have any bad effect on trees, and its effect on the pest is acceptable. Rajabpour et al. (2008) examined the efficiency of two mineral oils called, Cipron and Medium oils, with different physical characteristics with 1% proportion on the citrus cottony scale. Results showed that both types of mineral oils significantly decreased the population of all nymphal instars and adult insects. Mahmoud et al. (2007) investigated the effect of mineral oil and Chlorpyrifos insecticide against soft sale and rodent pests in gardens. And the results showed that Chlorpyrifos-methyl was the most effective treatment for all months after spraying, the mean reduction percentage being 94.10, 91.63 and 92.00% while it gave the highest toxic effect after three months; 90.27, 87.84 and 89.73% reduction in infestation on pre-adult, adult and gravid female stages, respectively. Michaud et al. (2004) examined the toxicity level of used insecticides against natural enemies of the red armoured scale, *Aonidiella aurantii* in citrus gardens. Abdel-Moniem (2005) investigated the susceptibility of different varieties of wheat to soft scale and also used pesticides against this pest. Results showed that the mortality rate, after application of Cruiser insecticide, reached to 61.16% after four weeks. Also, maximum mortality in April reached to 88.4%, and the maximum increase in product reached to 3.7% compared to control. Dean et al. (1976) examined the effect of application of oil with Azinphos-methyl, Carbosulfan and Chlorobenzilate against soft sale and grapefruit armoured scale. Blank et al. (1997) investigated the best time for spraying against citrus soft scale with Buprofezin and Chlorpyrifos insecticides with 1 and 2% oil. Results indicated that oil spray in April and the application of insecticides with 1% oil was the best method. Cowles (2010) examined the effect of systemic pesticide, Dinotefuran, on the pine armoured scale in natural condition. He concluded that maximum intake of insecticide is made when it was sprayed on the tree trunk. Therefore, suitable condition for the activity of natural enemies will be provided.

Overall, the results showed that efficiency of Buprofezin insecticide was more than that of various insecticides on first nymphal instars of *P. aurantii* and the DG oil has the highest synergistic effect on Malathion insecticide. Due to the harmful effects of insecticides on the environment, application of this combination could have important role in reducing environmental pollution and pest resistance.
Acknowledgement
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