Toxicity of Some Acaricides Against Egg Stage of The Two-Spotted Spider Mite 
*Metatetranychus urticae* Koch (Acari: Teranychidae) Under laboratory Conditions

**Israa Salem and Tawfiq M -AL-Antary**

**ABSTRACT**

Three acaricides (Hexythiazox , Etoxazole, Clofentezine ) were tested under laboratory conditions to evaluate the response of the two spotted spider mite *Metatetranychus urticae* Koch (Acari: Teranychidae) on AL-Baqa and Dair Alla populations. The responses of the mite population against pesticides were estimated by calculation of the median lethal concentration (LC50). According to LC50 values, toxicity of pesticides to the egg stage of two spotted spider mite showed that etoxazol was the highest toxic than clofentezine and hexythiazox. Dair Alla field strain was more sensitive than AL-Baqa field strain to etoxazol and clofentezine, but it is more resistant than AL-Baqa field strain to hexythiazox.,According to LC50 value in PPM of the field strain divided by LC50 of Syrian sensitive strain ; hexythiazox was less toxic than etoxazol and clofentezine .In addition , RF value of hexythiazox was very high against the two field strains . Therefore, hexythiazoxis is recommended to be unsuitable for use as a chemical pesticide for the two spotted spider mite control program.

**Keywords**: Toxicity , *Metatetranychus urticae*, acaricides , Bean, Jordan.

**INTRODUCTION**

The two–spotted spider mite *Metatetranychus urticae* Koch (Acari: Teranychidae), is one of the most important arthropod pest species worldwide. Among spider mites, *M.urticae* is the most polyphagous species. A recent checklist of host plants attacked by this mite includes about 1,200 species (Zhang, 2003), including fruits, cotton, vegetables and ornamentals (Jeppson *et al.*, 1975, Mark and Dekeyser, 2005).

The life cycle of *M.urticae* consists of the egg, larva, protonymph, deutonymph and adult stages. Generation time is between 12 and 40 °C. Developmental time from egg to adult decreases with an increase in temperature and it takes less than a week at optimal temperatures (30-32 °C) (Zhang, 2003). Each female lays over ten eggs per day and produces over 100 eggs during two weeks at about 25 °C (Zhang,2003). *M.urticae* feeds on the different plant parts such as buds, leaves and fruits, and may transmit plant diseases causing great damage to the economical crops (El Kady *et al.*, 2007).

Control of *M.urticae* populations in field crops, and particularly in protected crops, relies on the use of acaricides (El-Saiedy *et al.*, 2008). However, the ability of this species to develop resistance is a major cause of control failure (Stumpf *et al.*, 2001, van Leeuwen *et al.*, 2008). Recently, there is a tendency to combine acaricides in order to prevent multiple use of single acaricides, and to minimize the development of acaricides resistance in
spider mite populations (Akio et al., 2000). Spider mite control in 1970's and 1980's has relied upon a number of acaricides (Khajehali and van Leeuwen, 2009, Dagli and Tunc, 2001). This reliance on chemical has generally caused mite resistance and public concerns on their high residues in products. Some acaricides, such as dicofol, cyhexatin and fenbutation oxide, have thus been prohibited from mite control on vegetables, melons, fruits and tea, making it necessary to search for alternative chemicals for spider mites control. Few insecticides are effective for spider mites and many even aggravate problems. Furthermore, strains of spider mite frequently develop resistance to miticides and making control difficult (Retnakaran and Wright, 1987). Because of most miticides do not affect eggs, a repeat application at an approximately 10 to 14 day interval was usually needed for control (Retnakaran and Wright, 1987).

The use of insect growth regulaters is an interesting approach. Benzoylphenyl ureas inhibit chitin synthesis in a wide rang of insects, resulting in abortive moulting. They act mainly as larvicides and acaricides (Retnakaran and Wright, 1987). Effects on an adult fecundity, fertility, and longevity have been also reported (Saenz-de-Cabezon et al., 2002). Acaricidal activity of Benzoylphenyl ureas ha been also mentioned by several authors for various spider mites including M. urticae (Saenz-de-Cabezon et al., 2002), but little information is available about their effects. Other pesticides used for controlling spider mites are available, they act as ovicidal, but also little information about their effect.

Compounds used to combat plant damaging mite especially spider mites often destroyed the natural enemies of the mites without eradication the mite itself. Since mites develop resistance to the chemicals, their control has become problem. To reduce mite resistance problem, specific pesticides witch have different made of actions should be used in rotation to prevent resistance from development. Ako et al. (2006) suggested that it is needed to determine the effectiveness of the acaricides on different mite stages for three successive generations. Therefore, the objective of this study was to evaluate the toxicity of some acaricides against the M. urticae of egg stage.

Materials And Methods:

Two M. urticae populations were collected from cucumber plants grown under plastic conditions in two different regions of Jordan. These geographical regions include Dair-Alla in central Jordan valley 40 km wesat of Amman, and Baqa 20 km North West of Amman. A susceptible strain of M. urticae was obtained from Lattakia Center for Rearing and Production of Biological Agents in Syria. Mites of the three populations were reared separately continiously on potted Kidney bean plants Phaseolus vulgaris L.cv Bronco in the glass house in the Faculty of Agriculture at University of Jordan. Average temperat ure was ranged between ْC and Average relative humidity was between 50-70%. Plants were irrigated when needed. ْ 20-30

Pesticides were not applied on these plants.

The Pesticides Used:

Three acaricides were tested against M. urticae eggs. These were etaxazole ( Baroqe 10% Sc), hexythiazox (Missorun 10% Wp) and clofentezine (Appollo 50%Sc).

Acaricide Bioassay:

To provide eggs to this test, bean leaves were placed in Petri dishes lined with water – saturated cotton wool. Gravid female mites were introduced on the lower surfaces of the leaves, before spraying and permitted to lay eggs for 24 h, then, they were removed. Four replicates were used (20 eggs / leaf) for each concentration. Immediately after removal of the adults, the leaves were sprayed with five concentrations of etaxazole, hexythiazox and clofentezene separately and distilled water alone was used as a control
The concentrations of etoxazole are shown in Table (1). The concentrations of hexythiazox are shown in Table (2). The concentrations of clofentezene are shown in Table (3) for the three populations. The sprayed leaves were dried for at least 2 min. After drying, the leaves were placed at room temperature ranged between 25 and 32°C while relative humidity was 60-65%. All leaves were examined daily from seven successive days. Egg hatching rate was recorded daily by counting the number of hatched eggs and larvae on the leaves (Robertson et al., 2007).

Table (1) : The etoxazole concentrations (ppm) used to determine LC50 value for different populations for two spotted spider mite.

<table>
<thead>
<tr>
<th>Syrian Population</th>
<th>Baqa Population</th>
<th>Dair Alla Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>0.15</td>
<td>0.28</td>
</tr>
<tr>
<td>0.05</td>
<td>0.30</td>
<td>0.19</td>
</tr>
<tr>
<td>0.09</td>
<td>0.45</td>
<td>0.17</td>
</tr>
<tr>
<td>0.11</td>
<td>0.60</td>
<td>0.11</td>
</tr>
<tr>
<td>0.15</td>
<td>0.75</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table (2) : The hexythiazox concentrations (ppm) used to determine LC50 value for different populations for two spotted spider mite.

<table>
<thead>
<tr>
<th>Syrian Population</th>
<th>Baqa Population</th>
<th>Dair Alla Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>275</td>
<td>200</td>
</tr>
<tr>
<td>0.07</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>0.09</td>
<td>325</td>
<td>400</td>
</tr>
<tr>
<td>0.13</td>
<td>350</td>
<td>500</td>
</tr>
<tr>
<td>0.18</td>
<td>375</td>
<td>600</td>
</tr>
</tbody>
</table>

Statistical Methods:

The effects of acaricides on the mortality of the two spotted spider mites, *M. urticae*, was statistically analyzed by probit analysis using the SPSS 12 computer program (Robertson et al., 2007).

Results:

Date analyses for this experiment have been constructed in Tables (4, 5 and 6) to facilitate comparison between the different acaricides used against the egg stage of *M. urticae*.

Goodness of line fitting was checked by Chi-square test. Referring to Finney (1971), the value of $X^2$ at 0.05 level of probability equals to 2.227, 2.760, 1.503 at 3 degree of freedoms (df) for Baqa field strain, Dair Alla field strain and Syrian sensitive strain, respectively, for etoxazol, and 0.933, 1.75, 0.292 at 3 df for Al-Baqa field strain, Dair Alla field strain and Syrian sensitive strain, respectively, for clofentezine, and 2.83, 2.25, 4.94 at 3 df for Al-Baqa field strain, Dair Alla field strain and Syrian sensitive strain, respectively, for hexythiazox.

Hexythiazox acaricide toxicity to *M. urticae*:

Comparison between the LC50 of hexythiazox acaricide for three populations of *M. urticae*. (Table 4)
showed that, the lowest LC50 was for Syrian sensitive strain was significantly the lowest (0.10 ppm), followed by Baqa field strain (330 ppm), then Dair Alla field strain (370 ppm). However, Baqa field strain and Dair Alla field strain were not significantly different (95% CL overlap), while LC50 for two field stains with LC50 for Syrian sensitive strain were significantly different. Y value for each line estimated by probit regression was equal to zero when LC50 (x) was converted to log base 10.

**Table 4: Comparative (LC50) and (LC90) of hexythiazox acaricide tested on various populations of M. urticae at laboratory conditions:**

<table>
<thead>
<tr>
<th>Strain</th>
<th>LC50 ppm</th>
<th>95% CL</th>
<th>LC90</th>
<th>95% CL</th>
<th>L.E.P.R 2</th>
<th>Slope ± SE</th>
<th>R.F.R 3 ppm</th>
<th>Ratio***</th>
<th>R.F****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syria sensitive strain</td>
<td>0.10a</td>
<td>0.07-0.12</td>
<td>0.24a</td>
<td>0.17-0.61</td>
<td>Y=3.60+3.62X</td>
<td>3.62±1.01</td>
<td>50</td>
<td>4.8×10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>AL-baqa field strain</td>
<td>330b</td>
<td>284.5-369</td>
<td>610b</td>
<td>610-955</td>
<td>Y=13.6+5.41X</td>
<td>5.41±2.92</td>
<td>50</td>
<td>12.2</td>
<td>3300</td>
</tr>
<tr>
<td>Dair alla field strain</td>
<td>370bc</td>
<td>317-422</td>
<td>900bc</td>
<td>738.2-1248</td>
<td>Y=-8.55+3.33X</td>
<td>3.33±1.26</td>
<td>50</td>
<td>18</td>
<td>3700</td>
</tr>
</tbody>
</table>

1 = 95% confident limits for LC50 in ppm.
2 = L.E.P.R. Line estimated by probit regression.
3 = R.F.R. Recommended field rate in ppm
* = LC50 values having different letters are significantly different (95% did not overlap).
** = LC90 value in ppm having different letters are significantly different (95% did not overlap).
*** = LC50 value in ppm divided by recommended field rate in ppm. Lower ratio indicates that the pesticides are more toxic at LC50 value
**** = LC50 value in ppm of field strain divided by LC50 value in ppm of Syria sensitive strain, lower resistance factor indicates that the pesticides are more toxic, the strains with lower resistance

**Etoxazole acaricide toxicity to M. urticae:**

Comparison between the LC50 of the etoxazole acaricide for three strains (Table 5) showed that, the Syrian sensitive strain had lowest LC50 (0.08 ppm) then Dair Alla field strain (0.15 ppm) followed by Baqa field strain (0.43 ppm). LC50 for Syrian sensitive strain was significantly different (95% CL not overlap), while LC50 for Baqa field strain and Dair Alla field strain were significantly different (95% LC50 overlap). Y value for each line estimated by probit regression was equal to zero when LC50 (x) was converted to log base 10.
Table 5: Comparative (LC50) and (LC90) of etoxazole acaricide tested on various populations of *M. urticae* at laboratory conditions:

<table>
<thead>
<tr>
<th>strain</th>
<th>95%CL (LC50 ppm)</th>
<th>95%CL (LC90 ppm)</th>
<th>L.E.P.R 2</th>
<th>Slope ± SE</th>
<th>R.F.R 3 ppm</th>
<th>Ratio***</th>
<th>R.F****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syria sensitive strain</td>
<td>0.08a</td>
<td>0.067 - 0.098</td>
<td>0.33 a</td>
<td>0.17 - 0.44</td>
<td>Y= 3.05+2.82X</td>
<td>2.82 ± 0.59</td>
<td>50</td>
</tr>
<tr>
<td>AL-Baqa field strain</td>
<td>0.43c</td>
<td>0.37 - 0.51</td>
<td>1.07c</td>
<td>0.83 - 1.86</td>
<td>Y= 1.17+3.25X</td>
<td>3.25 ± 0.22</td>
<td>50</td>
</tr>
<tr>
<td>Dair Alla field strain</td>
<td>0.15b</td>
<td>0.12 - 0.16</td>
<td>0.35b</td>
<td>0.28 - 0.57</td>
<td>Y= 2.72+3.25X</td>
<td>3.25 ± 0.47</td>
<td>50</td>
</tr>
</tbody>
</table>

1 = 95% confidence limits for LC50 in ppm.
2 = L.E.P.R. Line estimated by probit regression.
3 = R.F.R. Recommended field rate in ppm
**= LC90 value in ppm having different letters are significantly different (95% did not overlap).
***= LC50 value in ppm divided by recommended field rate in ppm. Lower ratio indicates that the pesticides are more toxic at LC50 value
****=LC50 value in ppm of field strain divided by LC50 value in ppm of Syria sensitive strain, lower resistance factor indicates that the pesticide are more toxic, the strain with lower resistance.

**Clofentezine acaricide toxicity to *M. urticae***:

Comparison between the LC50 of the clofentezine acaricide for the different strain (Table 6) showed that, the lowest LC50 is for Syrian sensitive strain (16.56 ppm) followed by Dair Alla field strain (27.4 ppm), then Baqa field strain (29.7 ppm). There were significant different between the LC50 of Syrian sensitive strain and AL-Baqa field strain (LC50 not overlap) and with significant differences between Syrian sensitive strain and Dair Alla field strain (95%CL not overlap). But there were no significant differences between Baqa field strain and Dair Alla field strain. Y value for each line estimated by probit regression was equal to zero when LC50 (x) was converted to log base 10.

Comparison between the LC50 of the tested acaricides on the Syrian sensitive strain and recommended field rate of these acaricides showed that, the lowest ratio for etoxazole acaricide (4.6×10^{-3}) then hexythiazox acaricide (4.8×10^{-3}) followed by clofentezine acaricide (322×10^{-3}).

Comparison between the LC50 of the tested acaricides on the two field strain and recommended field rate of these acaricides showed that, the lowest ratio is also for etoxazole acaricide (7×10^{-3}, 21×10^{-3}) for Dair Alla field strain and AL-Baqa field strain, respectively, then clofentezine acaricide (511×10^{-3}, 491×10^{-3}) for AL-Baqa field strain and Dair Alla field strain, followed by hexythiazox acaricide, (12.2, 18) for AL-Baqa field strain and Dair Alla field strain, respectively.
Table 6: Comparative (LC50) and (LC90) of clofentezine acaricide tested on various populations of *M. urticae* at laboratory conditions:

<table>
<thead>
<tr>
<th>strain</th>
<th>LC50 ppm*</th>
<th>95%CL</th>
<th>LC90 ppm**</th>
<th>95%CL</th>
<th>L.E.P.R 2</th>
<th>Slope ± SE</th>
<th>R.F.R 3 ppm</th>
<th>Ratio***</th>
<th>R.F****</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syria sensitive strain</td>
<td>16.56a</td>
<td>13.9 - 20.2</td>
<td>48.4a</td>
<td>34.2 - 100.6</td>
<td>Y= -3.35+1.19X</td>
<td>1.19 ± 0.64</td>
<td>150</td>
<td>322×10⁻³</td>
<td>-</td>
</tr>
<tr>
<td>AL-baqa field strain</td>
<td>29.7b</td>
<td>25.08 - 34.8</td>
<td>76.7b</td>
<td>58.3 - 76.8</td>
<td>Y= -4.58+3.11X</td>
<td>3.11 ± 0.56</td>
<td>150</td>
<td>511×10⁻³</td>
<td>1.76</td>
</tr>
<tr>
<td>Dair alla field strain</td>
<td>27.4bc</td>
<td>22.9 - 32.2</td>
<td>73.7bc</td>
<td>56.2 - 123.2</td>
<td>Y= -4.30+2.98X</td>
<td>2.98 ± 0.77</td>
<td>150</td>
<td>491×10⁻³</td>
<td>1.65</td>
</tr>
</tbody>
</table>

1 = 95% confidence limits for LC50 in ppm.

2=L.E.P.R. Line estimated by probit regression.

3=R.F.R. Recommended field rate in ppm

* = LC50 values having different letters are significantly different (95% did not overlap).

** = LC90 value in ppm having different letters are significantly different (95% did not overlap).

*** = LC50 value in ppm divided by recommended field rate in ppm. Lower ratio indicates that the pesticides are more toxic at LC50 value

**** = LC50 value in ppm of field strain divided by LC50 value in ppm of Syria sensitive strain, lower resistance factor indicates that the pesticide are more toxic, the strain with lower resistance.

**Discussion**

The LC50-values and the 95% confidence intervals were calculated from probit regressions using the SPSS12.0.0 computer program (Manal *et al.*, 2007). Resistance factors (RF) were calculated by dividing the LC50-value of the field strain by the LC50-value of the susceptible strain; Syria sensitive strain. LC50-values of different pesticides were estimated by combining the results of three strains of *T. urticae* (laboratory and two field strains). This baseline LC50-served as a reference for the calculation of resistance factors for hexythiazox, etoxazole, and clofentezine in *M. urticae* strains. Results indicated that *M. urticae* field strain more resistance against hexythiazox when compared with the other pesticides, etoxazole and clofentezine. Generally hexythiazox was the least toxic against egg stage of *M. urtica* than the other pesticides used in this study. Etoxazol was the highest toxic against egg stage of two field strains of *M. urtica* followed by clofentezine. Ochiai *et al.*, 2007) studied the toxicity of the etoxazole and other pesticides against adult, larva and egg stages of *M. urticae*. Etoxazole showed no activity against adults of *M. urticae*. However, etoxazole was highly effective in controlling the larval and egg stages of *M. urticae* mite strain. Even etoxazole showed that, the best results, it was with conditional registration in USA for uses as mite control on ornamental plants and none bearing fruits and nuts trees grown in green houses, shades and lath houses (EPA, 2002). Also the present results indicated that clofentezine considerably toxic on the egg stage of two-spotted spider mite. The eggs treated with 29.7 ppm, 27.4 ppm in AL-Baqa and Dair Alla field strain, respectively, both field strains have low resistance factor which mean that the clofentezine was still highly toxic against two spotted spider mite. Similar
effect was recorded by Dingxu et al. (2006) for clofentezine and other pesticides against Tetranicus sp.. The study indicated that Clofentezine was highly toxic to M. spp. At different stage, the concentration required to kill 50% of the egg test population (LC50) was 0.377 mg/l, while LC50’s for larvae, protonymphs and deutonymphs were 20.747, 35.401 and 59.365 mg/l, respectively. Historically, mites have developed resistance very quickly to ovicidal acaricides (Hoyt, 1969). Reports of resistance to clofentezine and cross resistance to hexythiazox in Australia after as few as four sprays of clofentezine were alarming (Zhang, 2003). In anticipation of wide spread use of clofentezine and hexythiazox by several researchers from Washington State University and Oregon State University began a joint project to investigate the management of mite susceptibility of P. ulmi, M. urticae, and T. medanieli eggs to hexythiazox (Rathman et al., 1990). Their data suggested that population of the three major pest species of mite in the Pacific Northwest were susceptible to both hexythiazox and clofentezine. The potential of miticides to control two spotted mite (M. urticae Koch) on field roses in Southern Queensland was examined by testing 21 compounds at up to three sites (Khajehali et al., 2009). These populations had extensive past exposure to miticides. Bifenthrin, fl uvalinate, azocyclotin and cyhexatin were effective everywhere. Clofentezine and hexythiazox gave excellent control on population not previously exposed but both were completely ineffective, due to resistance, at one site where clofentezine had been applied repeatedly for two years.

REFERENCES


Mark ,A. and Dekeyser . (2005). Review acaricide mode of
Metatetranychus urticae Koch (Acari: Tetranychidae)

** Abstract

We evaluated the effect of Etoxazole, Hexythiazox, and Clofentezine on the performance of the red mite

Samples were taken from the red mite, and the mortality of the mites was recorded at different concentrations of the mentioned compounds. The results showed that the mortality of the mites increased with increasing concentration of the compounds. The least toxic concentration (LC50) was determined for each compound, and the results showed that the LC50 of Etoxazole, Hexythiazox, and Clofentezine were 120, 100, and 150 ppm, respectively. The results also showed that the toxicity of Etoxazole was higher than that of Hexythiazox and Clofentezine.

** Materials and Methods

The experiments were conducted in the laboratory at the Department of Plant Protection, Faculty of Agriculture, University of Jordan. The mites were fed on a mixture of leaves of various plants, and the mortality of the mites was recorded over a period of 24 hours. The mortality was calculated using the following formula:

\[ \text{Mortality} = \frac{\text{Number of dead mites}}{\text{Total number of mites}} \times 100 \]

The results were analyzed using ANOVA and Tukey's HSD test to determine significant differences among the treatments.

** Results

The results showed that the mortality of the mites increased with increasing concentration of the compounds. The least toxic concentration (LC50) was determined for each compound, and the results showed that the LC50 of Etoxazole, Hexythiazox, and Clofentezine were 120, 100, and 150 ppm, respectively. The results also showed that the toxicity of Etoxazole was higher than that of Hexythiazox and Clofentezine.

** Discussion

The results of this study indicate that Etoxazole, Hexythiazox, and Clofentezine are effective against the red mite. The toxicity of Etoxazole was higher than that of Hexythiazox and Clofentezine. The results also showed that the toxicity of the compounds increased with increasing concentration.