Effects of Sublethal Dose of Different Pesticides on the Two-spotted Spider Mite "Tetranychus urticae Koch" and its Predatory Mites under Greenhouse Conditions

Saeed Alzoubi and Sultan Çobanoğlu

Department of Plant Protection, Faculty of Agriculture, Ankara University, 06110 Dışkapı, Ankara, Turkey

Abstract: Sub lethal dose of three commonly used pesticides in Turkey were evaluated on the Two-spotted Spider Mite (TSSM) Tetranychus urticae Koch and its predatory mites, (Phytoseiulus persimilis A.H, Amblyseius californicus McGregor) under greenhouse conditions during the season in 2007. The three pesticides (hexythiazox: A selective acaricide; bifenthrin: A pyrethroid insecticide-acaricide and dimethoate: An organophosphate insecticide-acaricide) at of one-third the recommended field concentration as a low dosage were applied directly after releasing the predators to compare with pesticides without predators or with predators alone for controlling TSSM population. Additionally, IOBC toxicity criteria was used to evaluate the toxicity of tested pesticides against predators. Greenhouse results indicated that the toxicity of bifenthrin against P. persimilis was with harmful effects but in case of A. californicus, it was moderately harmful. While the toxicity of dimethoate against tested predators was moderately harmful but hexythiazox toxicity against tested predators was with harmless effects. The efficacy of the combination of hexythiazox, bifenthrin combined with predators (P. persimilis or A. californicus) and dimethoate with A. californicus on TSSM population can be effectively controlled the TSSM on cucumber plant when compared to the predatory mites alone. But on other hand, hexythiazox and dimethoate could not controlled the TSSM population properly. While the functions of P. persimilis seemed to be influenced negatively with dimethoate effect therefore, P. Persimilis combined with dimethoate can not provided rapidly and satisfactory control. Therefore, the sub lethal effects of pesticides may be used to controlling the TSSM population combined with predators in IPM programmes.

Key words: Sub lethal effects · IOBC toxicity criteria · IPM · Tetranychus urticae · Phytoseiulus persimilis · Amblyseius californicus

INTRODUCTION

Pesticides may destroy or harm natural enemies following exposure by contact, ingestion or less commonly by respiration. They may also affect natural enemies indirectly by killing or contaminating their hosts or prey [1]. Natural enemies are usually more susceptible to the effects of pesticides than their plant-feeding hosts or prey owing to their generally smaller size, searching habits, usually less-developed enzyme-based detoxification systems. For a given population of natural enemies exposed to a pesticide over a range of doses, some natural enemies will generally die within a relatively short period (e.g., 48 hours) while others may survive beyond that period [2]. There are many different ways to test the effect of a pesticide or other compound on predatory mites, beginning with a slide-dip study and progressing in complexity to a field-scale study [3]. Sublethal effects are those effects that occur in the pesticide-exposed survivors. The behavioural or physiological nature of sublethal effects on natural enemies tends to be fundamentally similar, although less severe, compared with that of lethal effects [4].

Farmers need to control a whole range of pests, not just one and crop diseases as well. Often the most effective control programs are based on a combination of biological and chemical control. For this to be possible, scientists must select strains and species of natural enemies which are resistant to chemicals. They must also identify those chemicals which are compatible with natural enemies [5].

The Two-spotted Spider Mite (TSSM) is a common cause of crop damage in greenhouses in Turkey. It is difficult to be controled with chemicals. Most species have become resistant and the mite hides underneath leaves where sprays and powders cannot reach [6]. A
species of predatory mite, *Phytoseiulus persimilis*, is being mass reared in Turkey as a biological control agent of two-spotted spider mite in vegetables. This predatory mite was recorded in natural colonies along the Turkish Mediterranean coast [7]. *A. californicus* is also found naturally in Turkey and was first recorded in 2001 on strawberry, peach, bean and pepper plants [8].

The aim of this study is to evaluate the effect of three pesticides (sublethal dose) on *T. urticae*, *P. persimilis* and *A. californicus* populations as part of a mite management program and to define the toxicity of used pesticides against predators according to IOBC toxicity criteria under greenhouse conditions.

**MATERIALS AND METHODS**

**Chemicals:** Two insecticides-acaricides, bifenthrin and dimethoate and a selective acaricide and mite growth regulator hexythiazox, were used in this experiment. These pesticides are commonly used to control spider mites and insects on vegetable crops in Turkey. The recommended field concentration of bifenthrin is 0.06 g a.i. L⁻¹ (60 ppm) (Talstar® 10 EC, 100 g a.i. L⁻¹, Bayer): Pyrethroid insecticide-acaricide. The recommended field concentration of dimethoate is 0.45 g a.i. L⁻¹ (450 ppm) (Polga® EC, 400 g a.i. L⁻¹, Hektos): Organophosphate insecticide-acaricide. The recommended field concentration of hexythiazox is 0.05 g a.i. L⁻¹ (50 ppm) (Twister® 5 EC, 50 g a.i. L⁻¹, Hektos). The experimental concentration of the pesticides was one-third of the recommended field concentration i.e. bifenthrin 0.02 g a.i. L⁻¹ (20 ppm), dimethoate 0.15 g a.i. L⁻¹ (150 ppm) and hexythiazox 0.017 g a.i. L⁻¹ (17 ppm).

**Source of mites:** Tetranychus urticae Koch was reared on bean plants (*Phaseolus vulgaris* cv. Barbonia) at 25±1°C and 65±10% RH under a 16-h light regime. Clean plants were grown in a climate room (same regime) until they were 2-weeks old and were subsequently added biweekly to the spider mite culture. The predatory mites, *Phytoseiulus persimilis* A.H and *Amblyseius californicus* McGregor were reared at 25°C on detached bean leaves infested with two-spotted spider mites. These leaves were put on an inverted flower pot in a water-containing tray covered with a Plexiglas container. Some 2-3 leaves from the spider mite culture were added to the cultures biweekly. *P. persimilis* A.H, Turkish strain, was obtained from fields in Hatay, Turkey. *A. californicus* McGregor, Spical®, was obtained from Koppert BV and reared on bean leaves with spider mites.

**Greenhouse trials:** Cucumber plants (*Cucumis sativus* L.) were used in the greenhouse as host plant. Cucumber seedlings were prepared for planting and then transferred to the greenhouse. Blocks of plants were separated from each other to prevent plants touching and mites moving between blocks and were surrounded with cloth barricades. Plants were infested on 01 April 2007 with TSSM (30 females/plant) when they became mature to the 4 real leaves phase. Predatory mites (*P. persimilis* A.H or *A. californicus* McGregor) were released i.e. 4 predatory females per plant, after 17 days from infestation with TSSM. Thereafter, pesticides were applied with a hand sprayer seven days after predators release. Leaf samples were taken from all blocks by the interval of 7, 14 and 21 days from application of pesticides and also before releasing and application. The number of predatory mites and also the egg, immature and adult stages of TSSM were counted on an area of 8 cm² of cucumber leaf in laboratory. Four leaves one from each plant (middle part), were sampled from each block. The corrected efficacy percent was calculated according to Sun-Shepard formula [9]:

\[
\text{Corrected efficacy} \% = \frac{\text{Change in treated plot population}}{\text{Change in control plot population}} \times 100 \text{ where:}
\]

- Population in control plot after treatment
- Population in control before treatment

\[
\text{Change in control} = \frac{\text{Population in control plot after treatment}}{\text{Population in control plot before treatment}} \times 100
\]

\[
\text{Change in treated} = \frac{\text{Population in treated plot after treatment}}{\text{Population in treated plot before treatment}} \times 100
\]

The classification of the side-effect of a pesticide was evaluated according to IOBC category (International Organization for Biological Control) for field and semi field tests against natural enemies where, 0-50% mortality is harmless or slightly harmful (N), 51-75% mortality is moderately harmful (M) and >75% mortality is harmful (T) [10].

The greenhouse experiments were designed in twelve blocks with 4 plants in each block. Six blocks containing pesticides and predatory mites (*P. persimilis* A.H or *A. californicus* McGregor), three blocks containing TSSM and pesticides without predatory mites and two blocks containing TSSM and predatory mites without pesticides and the last block was designed as a control block.
(TSSM+water). The average temperature and relative humidity were 23.05°C and 51% during overall trial period, respectively. Data were analyzed with ANOVA by using the computer program Cohort Software and means were separated by the aid of Duncan’s Test.

RESULTS

Effect of dimethoate: Results in Table 1 show that, the population of predators decreased and influenced by dimethoate effect which caused 75 and 66.66% mortality for *P. persimilis* and *A. californicus*, respectively. The efficacy of *A. californicus* with dimethoate gradually had increased over time, therefore, density of TSSM decreased and also caused decreasing the population of *A. californicus* (Fig. 1). While the efficacy of *P. persimilis* with dimethoate against TSSM population increased from 75.34 to 85.30% and then no significant increase was recorded. The efficacy of predators with dimethoate had showed significant difference when compared with dimethoate alone over time. Also, there was significant difference within the treatments which involved dimethoate with predators (Table 2).

Effect of bifenthin: The effect of bifenthin was very toxic to *P. persimilis* and caused 90.90% mortality and then was not observed during the test time. While the toxicity of bifenthin against *A. californicus* caused 52.94% mortality and then population of *A. californicus* was observed (Table 1) despite the low density of TSSM population, which remained under the influence of bifenthin effectiveness (Fig. 2). The efficacy of bifenthin against TSSM population showed no significant difference when compared with the treatments which involved predatory mites with bifenthin for 14 or 21 days after application (Table 2).

Table 1: Mean density and mortality ratio (%) of predatory mites for treatments of pesticides on an area of 8 cm² of cucumber leaf and classification of pesticides toxicity according to IOBC category

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Before applied</th>
<th>3 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>Mortality (%) (for third day)</th>
<th>IOBC category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethoate+ <em>P. persimilis</em></td>
<td>3.00</td>
<td>0.75</td>
<td>0.50</td>
<td>0.75</td>
<td>1.5</td>
<td>75.00</td>
<td>M</td>
</tr>
<tr>
<td>Dimethoate + <em>A. californicus</em></td>
<td>3.00</td>
<td>1.00</td>
<td>0.75</td>
<td>1.00</td>
<td>0.5</td>
<td>66.66</td>
<td>M</td>
</tr>
<tr>
<td>Bifenthin+ <em>P. persimilis</em></td>
<td>3.75</td>
<td>0.50</td>
<td>0.00</td>
<td>0.00</td>
<td>0.0</td>
<td>90.90</td>
<td>T</td>
</tr>
<tr>
<td>Bifenthin+ <em>A. californicus</em></td>
<td>4.25</td>
<td>2.00</td>
<td>3.00</td>
<td>1.00</td>
<td>0.0</td>
<td>52.94</td>
<td>M</td>
</tr>
<tr>
<td>Hexythiazox+ <em>P. persimilis</em></td>
<td>3.25</td>
<td>2.50</td>
<td>4.00</td>
<td>0.50</td>
<td>0.0</td>
<td>23.00</td>
<td>N</td>
</tr>
<tr>
<td>Hexythiazox+ <em>A. californicus</em></td>
<td>3.50</td>
<td>2.75</td>
<td>3.00</td>
<td>1.25</td>
<td>0.0</td>
<td>21.40</td>
<td>N</td>
</tr>
</tbody>
</table>

Table 2: Mean of corrected efficacy (±SE) for greenhouse treatments against TSSM population

<table>
<thead>
<tr>
<th>Treatment</th>
<th>7 days after application</th>
<th>14 days after application</th>
<th>21 days after application</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. persimilis</em>+ dimethoate</td>
<td>75.34±1.04d</td>
<td>85.50±0.79c</td>
<td>85.32±1.17d</td>
</tr>
<tr>
<td><em>A. californicus</em>+dimethoate</td>
<td>80.46±1.00c</td>
<td>90.49±0.61b</td>
<td>94.50±1.00b</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>56.00±1.51e</td>
<td>37.96±3.46d</td>
<td>31.55±2.49f</td>
</tr>
<tr>
<td><em>P. persimilis</em>+ bifenthin</td>
<td>95.41±1.06a</td>
<td>99.62±0.21a</td>
<td>99.82±0.17a</td>
</tr>
<tr>
<td><em>A. californicus</em>+ bifenthin</td>
<td>92.89±0.27a</td>
<td>99.48±0.17a</td>
<td>99.83±0.16a</td>
</tr>
<tr>
<td>Bifenthin</td>
<td>86.89±0.94b</td>
<td>98.70±0.27a</td>
<td>98.75±0.26a</td>
</tr>
<tr>
<td><em>P. persimilis</em>+ hexythiazox</td>
<td>94.28±0.67a</td>
<td>99.62±0.21a</td>
<td>99.81±0.18a</td>
</tr>
<tr>
<td><em>A. californicus</em>+ hexythiazox</td>
<td>88.77±0.85b</td>
<td>99.31±0.00a</td>
<td>99.83±0.16a</td>
</tr>
<tr>
<td>Hexythiazox</td>
<td>35.62±2.04g</td>
<td>42.05±2.15d</td>
<td>49.43±2.06e</td>
</tr>
<tr>
<td><em>P. persimilis</em></td>
<td>65.58±1.65e</td>
<td>84.99±0.84c</td>
<td>89.13±1.14c</td>
</tr>
<tr>
<td><em>A. californicus</em></td>
<td>66.66±1.51e</td>
<td>87.34±0.62c</td>
<td>88.27±0.79c</td>
</tr>
<tr>
<td>Control (water)</td>
<td>0.00±0.000</td>
<td>0.00±0.000</td>
<td>0.00±0.000</td>
</tr>
</tbody>
</table>

Statistic (Type: Split plot; LSD 0.05 = 2.88; Test: Duncan’s at P=0.05)

R2 = 0.99, df = 47

Means with different letters were significant different (P = 0.05, Duncan's Test)
Fig. 1: Mean density of TSSM and predatory mites population for treatments of dimethoate on an area of 8 cm² of cucumber leaf.

Fig. 2: Mean density of TSSM and predatory mites populations for treatments of bifenthrin on an area of 8 cm² of cucumber leaf.

**Effect of hexythiazox:** Density of predators population (P. persimilis and A. californicus) was not significantly affected for 3 days after the application of hexythiazox which caused 23 and 21.4% mortality sequentially (Table 1). Obviously, there was a complete and an energetic (synergistic) effect between the predators and hexythiazox against TSSM population and significant difference was found between the efficacy of predators with hexythiazox and the efficacy of hexythiazox alone or the efficacy of predators alone (Table 2).

The efficacy of predators with hexythiazox was effective to TSSM population over time. The decrease in density of TSSM population was accompanied by gradual decrease in the density of predators population (Fig. 3).

**Comparison of treatments against TSSM:** The efficacy of hexythiazox and bifenthrin combined with predators (P. persimilis or A. californicus) on TSSM population showed significant difference (P<0.05) with the treatments which involved predatory mites or with dimethoate and hexythiazox alone. Additionally, there was a significant difference between hexythiazox and dimethoate efficacy when compared with the predators efficacy during the test.
time. On the other hand, there was significant difference within the treatments which involved dimethoate with predators and also significant difference was found with hexythiazox and bifenthrin combined with predators (Table 2 and Fig. 4).

**DISCUSSION**

Toxicological studies on predaceous mites of economic importance are mainly concerned with measuring the possible adverse effects of pesticides on these tiny creatures. Data on initial toxicity of pesticides are indispensable to the development of safe spray programs for integrated control in orchards, fields, or greenhouses. Several test methods have been designed for the evaluation of pesticides in this respect. Although such methods do not always allow an exact prediction of what will happen in the field, they will give a fair clue as to what risks can be expected. Those pesticides that prove absolutely harmless in laboratory...
tests are in all probability also harmless in the field. In case of doubt semi-field tests or field tests should provide the answers [11].

Our study was designed as a 'semi-field' trial primarily to assess compatibility of some pesticides with P. persimilis and A. californicus to control spider mites under a realistic greenhouse conditions with cucumber as the host plant. In this paper, we used low concentrations of various pesticides as direct effect after the release of predatory mites to control TSSM. From our results, we concluded that after the application of hexythiazox the predators, P. persimilis or A. californicus can effectively control the Two-spotted Spider Mite (TSSM) on cucumber as compared to the predatory mites alone or to hexythiazox alone which could not control the mite properly. While after the application of dimethoate, P. persimilis can effectively control the TSSM only first 7 days when compared to the predatory mites alone or to dimethoate. Thereafter, significant difference was found with the efficacy of predators alone. Verkerk reported that the sub lethal dose of pesticides on natural enemies may have behavioural and physiological effects such as attack ratio, handling time, rate of discovery, development time and reproduction [4]. Therefore, P. persimilis efficiency seems to influence with direct effect of dimethoate and caused negatively effects to predator functions.

However, the effects of A. californicus or P. persimilis had supported the hexythiazox effects but only A. californicus had supported the dimethoate effects and provided effective and rapidly controlling TSSM population when compared to the predatory mites alone or to dimethoate and hexythiazox. The partly of these results seem to agree with Richard and Campbell who reported that the integrated control of T. urticae using clofentezine in conjunction with P. persimilis was likely to be more effective than an approach based on chemical or biological measures alone [12]. In addition, Verkerk reported that the effects of pesticides on natural enemies are often negative; pesticides can sometimes enhance natural enemy function particularly, if they are selective against the pests or are used at low dosages [4]. Although, the effectiveness of phytoseiid predators for the biological control of spider mites on their host plants, the predators alone may not be able to maintain spider mite populations below the economic injury level for an extended period of time [13-15]. Therefore, biological control of spider mites can be accomplished by the selective use of pesticides that are more toxic to the pest species than to its natural enemies [14, 16, 17].

The effect of bifenthrin was effective to TSSM population. Therefore, no significant difference was found within bifenthrin treatments particularly, after 14 and 21 days and there was significant difference with predators alone.

The toxicity of bifenthrin was extremely toxic to P. persimilis, this was not so with A. californicus and also according to IOBC category, toxicity of bifenthrin against P. persimilis has harmful effects but to A. californicus has moderately harmful effects. While the toxicity of dimethoate against tested predators is moderately harmful but the toxicity of hexythiazox against tested predators is with harmless effects.

Kenneth et al. [18] found that abamectin, Gowan 1725, hexythiazox, horticultural oil, neem oil, pyridaben and spinosan residues caused no mortality to P. persimilis 1, 3, 7, or 14 days after application and that T. urticae mortality from hexythiazox and spinosan residues was not significantly greater than the control and bifenthrin and chlorfenapyr residues were toxic to P. persimilis [18]. Sato et al [19] reported that fenpyroximate, fenpropethrin, dimethoate, propargite, sulfur and benomyl were innocuous to A. californicus which used against TSSM on strawberry under field conditions [19].

Therefore, TSSM control can be successfully achieved by combining biological and chemical measures using chemicals with low dosage.

ACKNOWLEDGMENT

We would like to thank The Scientific and Technical Research Council of Turkey (TÜBİTAK) who supported this research. We would also like to thank Associate Prof. Dr. Cengiz Kazak and Ibrahim Çakmak for providing P. persimilis. We would like to thank Prof. Dr. M.Öktay Gürkan for his comment.

REFERENCES


