World Journal of Agricultural Sciences 3 (6): 764-770, 2007
ISSN 1817-3047

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

Saied 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 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 influnced negetively 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

Corresponding Author: Dr. Saied Alzoubi, Department of Plant Protection, Faculty of Agriculture, Ankara University, 06110 Dışkapı, Ankara, Turkey

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) (Poligor® EC, 400 g a.i. l^{-1}, Hektaş): 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}, Hektaş). 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. Barbunia) 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 in 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 intervel 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 cm^2 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]:

$$\text{Corrected efficacy \%} = \frac{\text{Change \% in treated plot} \pm \text{change \% in control plot population}}{100 \pm \text{Change\% in Control plot population}} = *100$$

Where:

$$\text{Change \% in control} = \frac{\text{Population in control plot after treatment} - \text{Population in control before treatment}}{\text{Population in Control plot before treatment}} *100$$

$$\text{Change \% in treated plot} = \frac{\text{Population in treated brfore treatment} - \text{Population in treated plot after treatment}}{\text{Population in treated plot before treatment}} *100$$

The classification of the side-effect of a pesticides 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 trail 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 bifenthrin: The effect of bifenthrin was very toxic to *P. persimilis* and caused 90.90% mortality and then was not observed during the test time. While the toxicity of bifenthrin 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 bifenthrin effectiveness (Fig. 2). The efficacy of bifenthrin agansit TSSM population showed no significant difference when compared with the treatments which involved predatory mites with bifenthrin 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 cm^2 of cucumber leaf and classification of pesticides toxicity according to IOBC category

Treatment	Before applied	3 days	7 days	14 days	21 days	Mortality (%) (for third day)	IOBC category (for third day)
Dimethoate+ *P. perisimilis*	3.00	0.75	0.50	0.75	1.5	75.00	M
Dimethoate + *A. californicus*	3.00	1.00	0.75	1.00	0.5	66.66	M
Bifenthrin+ *P. perisimilis*	3.75	0.50	0.00	0.00	0.0	90.90	T
Bifenthrin+*A. californicus*	4.25	2.00	3.00	1.00	0.0	52.94	M
Hexythiazox+ *P. perisimilis*	3.25	2.50	4.00	0.50	0.0	23.00	N
Hexythiazox+ *A. californicus*	3.50	2.75	3.00	1.25	0.0	21.40	N

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

	Corrected efficacy±s.e		
Treatment	7 days after application	14 days after application	21 days after application
P. persimilis+ dimethoate	75.34±1.04d	85.30±0.79c	85.32±1.17d
A.californicus+dimethoate	80.46±1.00c	90.49±0.61b	94.50±1.00b
Dimethoate	56.00±1.51e	37.96±3.46d	31.55±2.49f
P. persimilis+ bifenthrin	95.41±1.06a	99.62±0.21a	99.82±0.17a
A.californicus+bifenthrin	92.89±0.27a	99.48±0.17a	99.83±0.16a
Bifenthrin	86.89±0.94b	98.70±0.27a	98.75±0.26a
P. persimilis + hexythiazox	94.28±0.67a	99.62±0.21a	99.81±0.18a
A. californicus+hexythiazox	88.77±0.85b	99.31±0.00a	99.83±0.16a
Hexythiazox	35.62±2.04g	42.05±2.15d	49.43±2.06e
P. persimilis	65.58±1.65e	84.99±0.84c	89.13±1.14c
A. californicus	66.66±1.51e	87.34±0.62c	88.27±0.79c
Control (water)	0.00±0.00h	0.00±0.00e	0.00±0.00g
Statistic (Type: Split plot;	LSD 0.05 = 2.88	LSD 0.05 = 4.17	LSD 0.05 = 3.4
Test: Duncan's at P=0.05)	R2 = 0.99; d f = 47	R2 = 0.99; df = 47	5R2=0.99; df = 47

Means with different letters were significant different (P = 0.05. Duncan's Test)

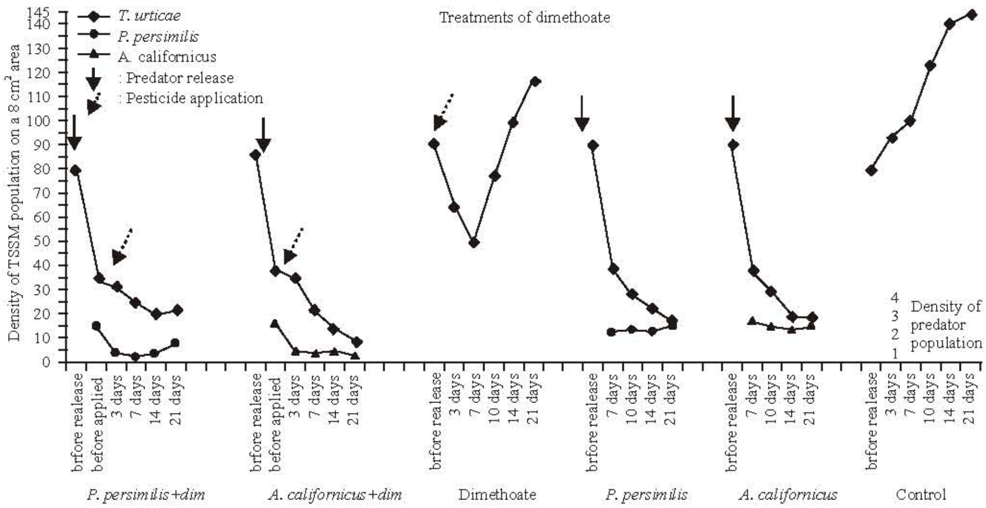

Fig. 1: Mean density of TSSM and predatory mites population for treatments of dimethoate on an area of 8 cm^2 of cucumber leaf

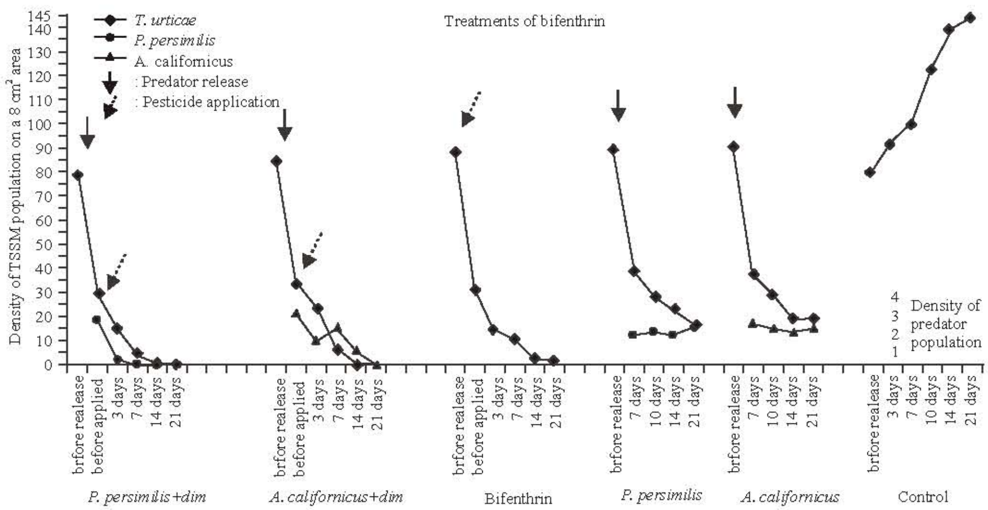

Fig. 2: Mean density of TSSM and predator mites populations for treatments of bifenthrin on an area of 8 cm^2 of cucumber leaf

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 hexythaizox and dimethoate efficacy when compared with the predators efficacy during the test

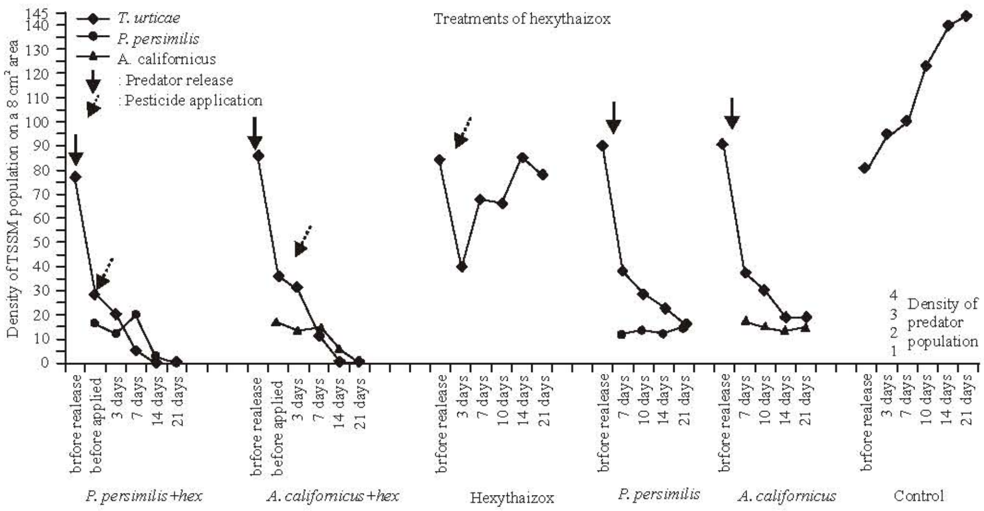

Fig. 3: Mean density of TSSM and predator mites populations for treatments of hexythiazox on an area of 8 cm^2 of cucumber leaf

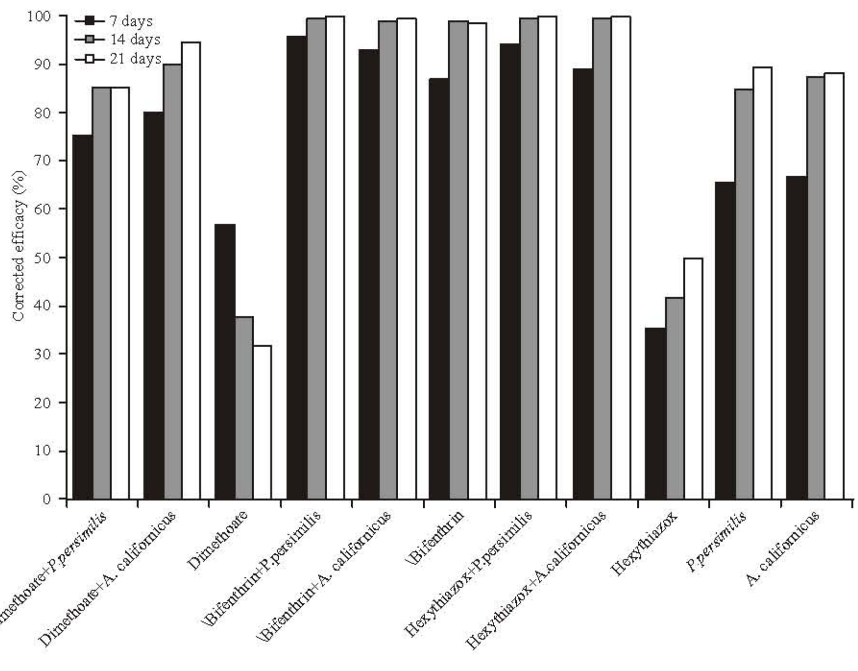

Fig. 4: Mean of efficacy (%) for greenhouse treatments against TSSM population

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 effecteve 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 spionosyn residues caused no mortality to *P. persimilis* 1, 3, 7, or 14 days after application and that *T. urticae* mortality from hexythiazox and spinosad 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, fenpropathrin, 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 (TÜBITAK) 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

1. Picone, C. and D.V. Tassel, 2002. Agriculture and Biodiversity Loss: Industrial Agriculture. Niles Eldredge (Ed.), Life on Earth: An Encyclopedia of Biodiversity, Ecology and Evolution, pp: 99-105. Reprinted with permission by ABC-CLIO, Santa Barbara, California.
2. Charlet, L., 1995. The Impact of pesticides on natural enemies. Midwest Biological Control News, Vol 2, Nr 2. http://www.entomology.wisc.edu/mbcn/fea202.html (accessed 02 May 2007).

3. Hassan, S.A. and P.A. Oomen, 1985. Testing the side effects of pesticides on beneficial organisms by OILB Working Party. In: Hussey N.W. and Scopes N. (Eds), Biological Pest Control–The Glasshouse Experience. Bland ford Press, Poole, Dorset, UK, pp: 145-152.
4. Verkerk, R., 2001. Farmers' Friends-recognition and conservation of natural enemies of vegetable pests: A field guide for Introduction extension staff and trainers in Zimbabwe. Biology Department, Imperial College of Science, Technology and Medicine, University of London SW7 2AZ, pp: 82-110.
5. Anonymous, Biological pest control. FFTC Newsletter 2001/133. http://www.agnet.org/library/vnl/133/nl133.pdf2001 (accessed 27May2007).
6. Ay, R., 2005. Determination of susceptibility and resistance of some greenhouse populations of *Tetranychus urticae* Koch to chlorpyrifos (Dursban 4) by the Petri dish-Potter tower method. J. Pest Sci., 78: 139-143.
7. Şekeroğlu, E. and C. Kazak, 1993. First record of *Phytoseiulus persimilis* A-H. (Acari: Phytoseiidae) in Turkey. Entomophaga, 38: 343-345.
8. Çakmak, I. and S. Çobanoğlu, 2006. *Amblyseius californicus* (McGregor, 1954) (Acari: Phytoseiidae), A new record for the Turkish fauna. Turk. J. Zool., 30: 55-58.
9. Püntener, W., 1981. Manual for field trials in plant protection. 2th. ed, Documenta Ciba-Geigy Agricultural Division. Basle, Switzerland.
10. Boller, E.F., H. Vogt, P. Ternes and C. Malavolta, 2006. Working Document on Selectivity of Pesticides (2005). Internal newsletter issued by the publication commission for the IOBC/wprs council and executive committee Issue Nr 40. http://www. iobc. ch/toolbox. html#5 (accessed 02 May 2007).
11. Hele, W. and W.P.J. Overmeer, 1985. Toxicological Methods. In: Helle W. and M.W. Sabelis (Eds.). Spider mites: Their biology, natural enemies and control, Netherlands, Elsevier, 1B: 183-188.
12. Richard, L. and C.A.M. Campbell, 1999. Biological, Chemical and Integrated Control of Two-spotted Spider Mite *Tetranychus urticae* on Dwarf Hops. Biocontrol Sci. Technol., 9: 467-473.
13. Field, R.P. and M.A. Hoy, 1986. Evaluation of genetically improved strains of *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae) for integrated control of spider mites on roses in greenhouse. Hilgardia, 54: 1-31.
14. Kim, S.S. and C.H. Paik, 1996a. Comparative toxicity of fenpyroximate to the predatory mite, *Amblyseius womersleyi* Schicha and the kanzawa spider mite, *Tetranychus kanzawai* Kishida. Appl. Entomol. Zool., 31: 369-377.
15. Ibrahim, Y.B. and T.S. Yee, 2000. Influence of sublethal exposure to abamectin on the biological performance of *Neoseiulus longispinosus* (Acari: Phytoseiidae). J. Econ. Entomol., 93: 1085-1089.
16. Hoy, M.A. and Y.L. Ouyang, 1986. Selectivity of the acaricides clofentezine and hexythiazox to the predator *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae). J. Econ. Entomol., 79: 1377-1380.
17. Zhang, Z.Q. and J.P. Sanderson, 1990. Relative toxicity of abamectin to the predatory mite, *Phytoseiulus persimilis* (Acari: Phytoseiidae) and two spotted spider mite (Acari: Tetranychidae). J. Econ. Entomol., 83: 1783-179.
18. Kenneth, W.C., E.L. Edwin and B.S. Peter, 2002. Compatibility of acaricide residues with *Phytoseiulus persimilis* and their effects on *Tetranychus urticae*. Am. Soc. Hortic. Sci., ASHS, 37: 906-909.
19. Sato, M.E., M.D. Silva., LR. Goncalves., M.F. Souza Filho and A. Raga, 2002. Differential toxicity of pesticides to *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *Tetranychus urticae* Koch (Acari: Tetranychidae) on strawberry. Neotrop. Entomol., 31: 449-456.