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Selectivity of Three Miticides to Spider Mite Predator, *Phytoseius plumifer* (Acari: Phytoseiidae) Under Laboratory Conditions

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Abstract

The predatory mite, *Phytoseius plumifer* (Canestrini & Fanzago), is one of the most abundant natural enemies and efficient predator of phytophagous mites in Iran. The miticides hexythiazox (Nisorun®, EC 10%), fenpyroximate (Ortus®, SC 5%), and abamectin (Vertimec®, EC 1.8%) were tested in the laboratory for their side effects on *P. plumifer*. The miticides were applied at the highest field recommended concentration (N) to detached leaves using a Potter Tower to deposit 2 mg spray solution per cm^2. Percent predator mortality was evaluated from the protonymph up to the adult stage including the first five days of the oviposition period. Analysis of data showed that the total effect values of hexythiazox were below the lower threshold, and thus it could be considered to be a harmless miticide to *P. plumifer*. In contrast, the total effects of fenpyroximate and abamectin were found harmful to the predatory mite at the highest field recommended concentrations.

Key words: *Phytoseius plumifer*, selectivity, side effect, miticide, predatory mite

INTRODUCTION

Spider mite management in agriculture crops depends on the frequent use of miticides. Increasing problems with resistance, reduction of predator densities, ecosystem disruption, environmental pollution, availability, and high cost of new miticides have led to a reevaluation of control strategies (Croft and Brown 1975; James 2002). Integrated pest management strategies, based on encouraging greater levels of biological control and reducing pesticide inputs, are being developed for the major arthropod pest like spider mites (James 2002). Biological control is an alternative pest management approach that has received increasing interest in recent years, which uses natural enemies to suppress mite pest populations below damaging levels. However, biological control of spider mites using the predaceous mite is effective only against low population densities of the pest (Pralavorio *et al*. 1985). When the population densities are too great, a miticidal treatment is needed to reduce the pest population before releasing beneficial control mites (Malezieux *et al*. 1992). Prospects for increasing levels of mite predation in agroecosystem appear to be positive, however, a critical factor in making crops more hospitable to predators will be the use of selective as well as narrow spectrum miticides. The European and Mediterranean Plant Protection Organization (EPPO) has developed a procedure for assessing the impact of pesticides on non-target organisms (EPPO/OEPP 1990). The protocol prescribed by the EPPO is based on the assumption that the pesticide found to be harmless to the biological control agents in laboratory bioassay will also be harmless to the same

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organism in the field (EPPO/OEPP 1990). Phytoseiid mites are excellent biological control agents for suppressing pest mite populations in a variety of crops and preventing yield losses (McMurtry and Croft 1997). The predatory mite, *Phytoseius plumifer*, is an important generalist indigenous predator of tetranychid mites and is widely found on various crops in Iran (Kamali *et al*. 2001; Hajizadeh *et al*. 2002), as well as in other countries (Rasmy and Elbanhawy 1974; Balevski *et al*. 1978; Castagnoli and Liguori 1985). Several miticides including hexythiazox, abamectin, and fenpyroximate, are currently used in Iran, however, the side effects of the miticides to key spider mite predators including *P*. *plumifer* are unidentified. Recognizing the side effects of the miticides on the predatory mite can help to enhance the success of biological control of spider mite pests.

MATERIALS AND METHODS

Origin and rearing of mites

The *Tetranychus urticae* Koch, originated from the greenhouse of the Department of Agricultural Zoology, Iran Plant Protection Research Institute, were reared on bean plants (*Phaseolus vulgaris* L.). *P*. *plumifer* originating from fig orchards near Pishva, Iran, were reared on detached fig leaves prepared as follows. Several detached leaves were placed underside up on a wet cotton wool layer in a Petri dish (80-mm diameter) with a base drilled centrally. The Petri dish was placed in a larger Petri dish (90-mm diameter) to provide continuous water for the cotton layer. The wet cotton wool prevented mites escape and maintained the leaf freshness for two weeks. *T*. *urticae* served as the prey of predatory mite. Mite cultures were maintained in separate climatic rooms at (25±2)°C, 60-70% relative humidity (RH) under a 16 L:8 D.

Preparation of the predator

The test was carried out with the most susceptible life stage, i.e., protonymphs (larvae are too fragile to be used). Protonymphs of uniform age were obtained according to the EPPO guideline (EPPO/OEPP 1990).

Miticides

The following three commercial miticides were tested at the highest field recommended concentration (N) in practice: abamectin (Vertimec®, EC 1.8%), fenpyroximate (Ortus®, SC 5%), and hexythiazox (Nisorun®, EC 10%). Single detached fig leaves were sprayed on the lower side with a potter spray tower (Burchard Manufacturing, UK) calibrated to achieve a wet deposit of 2 mg cm^{-2} and left to dry. Tap water was used as a control in all trials (Table 1).

Table 1 Miticides used in experiments

Common name	Brand name	Active ingredient	Field rate recommended (N) (mL L^{-1})
Hexythiazox	Nisorun, EC 10%	2 500 μg mL^{-1}	2.5
Abamectin	Vertimec, EC 1.8%	36 μg mL^{-1}	0.2
Fenpyroximate	Ortus, SC 5%	250 μg mL^{-1}	0.5

Toxicological tests

The experiments were carried out according to the EPPO guideline (EPPO/OEPP 1990) that combined lethal and sub-lethal effects as the total effects according to the formula proposed by Overmeer and van Zon (1982).

Tier I: study of residual initial activity After the spray residue was dried at day 0 of the experiment, predator protonymphs of uniform age were placed on the leaf arena using a fine brush and a surplus of spider mites was added as food. Sixty predator protonymphs (15 × 4 replicates) were used in each test unit. The mortality and escape of predators up to 5 days after the adult stage and the reproduction per female during the first 5 days of the adult stage were assessed. All dead and alive mites were counted, and dead mites were removed daily. Mites were considered dead when they failed to move after repeated gentle prodding with a brush. Predator eggs were counted and removed daily at the moment of the first oviposition day until the fifth oviposition day to assess the reproduction. All assessments were made with a stereomicroscope.

Tier II: persistence test An extra test with 3 days residue was performed to identify the duration of harmful persistence on detached leaves. In the test, detached leaves were sprayed with the highest recom-

mended field concentration of fenpyroximate and abamectin. After keeping detached leaves at rearing conditions for 3 days, bioassays were conducted as described for the residual initial toxicity test.

Analysis To avoid overestimating mortality, cumulative mortality was calculated by summing dead mites and dividing this number by the total number of alive and dead mites at each mortality assessment, excluding unaccounted escapees (Blumel *et al*. 1993). The escape rate was calculated as a portion of the number of mites present at the start of experiment. Mortality rates were corrected for the control mortality with the following formula (Abbott 1925):

$$M_a = (M_t - M_c)/(100 - M_c) \times 100\%$$

Where, M_a was mortality corrected according to Abbott (1925), M_t was mortality in treatment, and M_c was mortality in control.

The possible changes in the number of females present in the test units during the reproduction period were taken into account using the following formula:

$$R_{ry} = nE_{d1}/nF_{d1} + nE_{d2}/[(nF_{d1} + nF_{d2})/2] + nE_{d3}/[(nF_{d2} + nF_{d3})/2] + nE_{d4}/[(nF_{d3} + nF_{d4})/2] + nE_{d5}/[(nF_{d4} + nF_{d5})/2]$$

Where, d1 to d5 were examples for evaluation of adult days (d1, first adult day; d5, fifth adult day); R_{ry} was the reproduction in replicate number *y*; nE_{dx} was the number of eggs (in replicate number *y*) on day *x*; and nF_{dx} was the number of females (in replicate number y) on day *x*.

The mean values of the escape rate, the mortality rate, and the reproduction per female of the different treatments were statistically analyzed. The data were checked for normal distribution and analyzed by univariate analysis of variance (ANOVA, Duncan's test; SPSS 13.0 for windows). The data were transformed before analysis (square root).

The effect on the reproduction was determined by:

$$E_r = R_t/R_c$$

Where, E_r = Effect on reproduction; R_t = Reproduction in treatment; R_c = Reproduction in control.

The subsequent effect on the survival and the effect on reproduction were combined using the following formula (Overmeer and van Zon 1982):

$$E = 100\% - (100\% - M_a) \times E_r$$

Where, M_a = Mortality corrected according to Abbott (1925); E = Total effect.

Based on the total effects, the toxicity of miticides was evaluated through sequential decision-making scheme in the guideline (EPPO/OEPP 1990).

RESULTS

There was a significant difference in 7 d cumulative mortality effects of all three miticides at the field rates on *P. plumifer*. The mortality was the highest after the exposure to fenpyroximate and abamectin (100% mortality). In contrast, the mortality of *P. plumifer* exposed to dry residues of the field rate of hexythiazox did not show significant differences (Table 2).

The miticides also showed different effects on female fecundity of *P. plumifer*. Hexythiazox at the field rate did not show significant effect compared to the control (Table 2), while the effect of fresh residue of fenpyroximate and abamectin was significantly different from the control, where the mortality after one week was 100% (Tables 2 and 3). The results of 3-day old residue of fenpyroximate and abamectin on the mortality and fecundity of *P. plumifer* were similar to the fresh residue test of these miticides.

ANOVA yielded significant differences in the escape rates on predatory mites (Table 4). A mean comparison of escaped individuals showed that the escape rate from fresh residue of fenpyroximate was significantly lower than the control and hexythiazox.

The results of the total effects (E) of the product applications are listed in Table 3. According to EPPO

Table 2 Residual initial effect of the miticides on survival and fecundity of *P. plumifer*

Miticide	Concentration (mL L^{-1})	Mortality rates (mean±SE)	Total eggs/Female (mean±SE)
Control	-	-	6.26±0.29 a
Hexythiazox	2.5	3.35±2.05 a	6.01±0.26 a
Fenpyroximate	0.5	100.00 b	0.00 b
Abamectin	0.2	100.00 b	0.00 b

Means in the same column followed by different letter are significantly different ($P<0.05$, Duncan's test). The same as below.

Table 3 Total effect (E) and toxicity classes of the miticides for *P. plumifer* according to the EPPO classification

Miticide	Concentration (mL L^{-1})	Tier I (%, fresh residue)	Tier II (%, 3 days old residue)	Toxicity
Hexythiazox	2.5	7.21	Not tested	-
Fenpyroximate	0.5	100	100	+
Abamectin	0.2	100	100	+

- and +, harmless and harmful to *P. plumifer* in IPM programs, respectively.

Table 4 Escape rate of *P. plumifer* after exposure to fresh residue of miticides

Miticide	Concentration (mL L^{-1})	Escape rate (mean ± SE)
Control	-	25.00 ± 2.72 a
Hexythiazox	2.5	28.88 ± 1.57 a
Fenpyroximate	0.5	16.66 ± 4.30 b
Abamectin	0.2	21.66 ± 1.66 ab

classification of the toxic effects of the miticides, hexythiazox was harmless (E < 30) to *P. plumifer*, while abamectin and fenpyroximate at field rate were harmful (E > 99) to *P. plumifer*.

DISCUSSION

Selective pesticides that can be used to control pests without adversely affecting important natural enemies are urgently needed (Hassan *et al*. 1991). With introduction of new miticides into agricultural crops for controlling mite pests, it is required to consider the toxicity of these chemicals and to determine their selectivity to predatory mites that have paramount importance in integrated mite management (IMM) programs.

The present study indicated that hexythiazox was more selective to the predatory mite, *P. plumifer*, than two other miticides tested and it could be used in integrated control programs. Harmlessness of hexythiazox to the predatory mite is attributed by low mortality rate and non-effectiveness on fecundity (Table 2). Up to now, no information has been reported on the side effects of hexythiazox on *P. plumifer*. The nontoxic effect of hexythiazox on *P. plumifer* is consistent with that observed on other phytoseiids. A research demonstrated minimal harmful effects of hexythiazox to predatory mites including *Phytoseiulus persimilis* Athias-Henriot, *Amblyseius potentillae* (Garman), *Typhlodromus pyri* Scheuten, *Amblyseius finlandicus* (Oudemans), and *Amblyseius andersoni* (Chant) (Hassan *et al*. 1991). Oomen *et al*. (1991) and Blumel and Gross (2001) did not find any unfavorable effect of hexythiazox on the survival and fecundity of *P. persimilis* and thus classified it as harmless.

The stage of exposure to toxicant can make a large difference in terms of susceptibility. This is quite obvious with arthropod growth regulators that affect juveniles more than adults (Stark and Banken 1999). Harmless effect of hexythiazox on the predatory mite protonymphs may be owing to its growth regulating mode of action on moulting. Protonymph of these predators as well as the developmental stage of parasites (protected within their hosts) were not affected by this miticide (Hassan *et al*. 1991). Thus, exposure of protonymphs, as the most susceptible developmental stage, may result in inaccuracy in risk assessment. It may be true that protonymphs are not always the most susceptible stage, and thus we suggest that the side-effects of hexythiazox should be studied on other life stages, as discussed by Stark and Banken (1999) and Blumel *et al*. (2000).

In bioassays conducted with fresh residue of fenpyroximate and abamectin, our results showed poor selectivity of these miticides to the predatory mite, *P. plumifer* (E = 100%). The use of abamectin and fenpyroximate in an integrated pest management (IPM) system should therefore be carefully evaluated in field tests. Accordingly, we suggest that these miticides should not be used where *P. plumifer* are present.

Fenpyroximate used at recommended rate for practical use on the predatory mite was still persistent as spray deposit of 3 days old (E = 100%). Such data have not been reported previously for *P. plumifer* and other reports in the literature on the effect of fenpyroximate are mainly documented for other phytoseiid mites. In a study of the total effects of strawberry leaf surface residues of six acaricides including fenpyroximate on *P. persimilis* and *Galendromus occidentalis* (Nesbitt) (Irigaray *et al*. 2007), the results showed that fenpyroximate residues were not compatible with augmentative releases for at least 37 days after treatment. On the other hand, a study on the duration of fenpyroximate on phytoseiids has resulted in a different outcome. For example, van de Veire *et al*. (2001) observed that the duration of fenpyroximate on predatory mite *Amblyseius californicus* (McGregor) was short lived and favorable towards the predatory mite.

Similar to fenpyroximate, 3 days old residue of abamectin was also enormously toxic to *P. plumifer*, resulting in 100% mortality besides fresh residue test. Previous reports have shown varying degrees of abamectin toxicity to beneficial predatory mites. Negative effects of abamectin to *P. plumifer* were consistent in the findings of Noii *et al*. (2007) who reported that the exposure to abamectin residues had a great

total effect on *P. plumifer* (E=100%). Harmfulness of this miticide was also documented by Blumel and Hausdorf (2002) on *P. persimilis*. In addition, Kim *et al*. (2005) assessed the harmful effects of abamectin on adult female predator of *Amblyseius cucumeris* (Oudemans) and found that abamectin was very toxic. On the other hand, several reports have considered abamectin as an undisruptive chemical to use on phytoseiids in the IPM program. For example, Zhang and Sanderson (1990) suggested that since abamectin appears considerably more toxic to two spotted spider mites than *P. persimilis*, it can be used as a selective miticide in integrated spider mite control. Ibrahim and Yee (2000) also reported that although the reproductive performance of *Neoseiulus longispinosus* (Evans) was inferior to control, the longevity and the sex ratio of the eggs laid by the affected females treated by abamectin was not markedly affected, thus suggesting that the use of abamectin in IPM program will not be disruptive. Recently, Irigaray *et al*. (2007) indicated that abamectin on *G. occidentalis* and *P. persimilis* was short lived and slightly persistent, respectively.

Of fenpyroximate and abamectin, the differences in toxicities reported by various researches may owing to the differences in different life stages exposed to the miticides, in formulation and concentration of the miticide used, and in susceptibility variation among species. Consequently, phytoseiid species need to be examined independently to determine their compatibility with the two miticides in integrated mite management programs.

Our result showed that the fresh residue of fenpyroximate and abamectin caused lower escapes in the predatory mite *P. plumifer*, which may be a result of high mortality attributed to these miticides. In contrast, higher escapes occurred in the hexythiazox treatment (Table 4). It seems that accumulation of the predatory mites on the detached leaf surface could be the main reason of the escape from the surface. However, because of the high escape rates in the control, it was not possible to accurately estimate this parameter under laboratory conditions. Natural enemies like phytoseiid mites can move away from the treated leaves that are toxic to them and their population can be preserved from adverse effects. On the other hand, they cannot exert their beneficial activity on prey population. Ultimately, determining escapees, as the repellent effect of miticides, can have both advantages and disadvantages (Jansen 1999). Thus, we suggest that escape, i.e., a change in the behavior of the test mites, should be addressed at higher test tiers (Blumel *et al*. 2000).

CONCLUSION

Our results support the use of hexythiazox for spider mites management in the field to avoid disruption of mite biological control by the predatory mite *P. plumifer*. The miticides fenpyroximate and abamectin were potentially toxic to *P. plumifer*; however, further tests need to be carried out till an adequate assessment of their negative effects is made.

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