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Full Length Research Paper

Study on persistence tests of *miticides abamectin and fenproximate* to predatory mite *Phytoseiulus persimilis* (Acarina: Phytoseiidae)

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Economically, the most important species are the polyphagous pest two-spotted spider mite called *Tetranychus urticae* Koch and the predaceous phytoseiid mite called *Phytoseiulus persimilis* Athias-Henriot which is successfully used as biological control agent in a number of agricultural ecosystems. To evaluate the toxicity of the two current miticides (abamectin and fenpyroximate) on predatory mite *P. persimilis*, two persistence tests were carried out under laboratory conditions and with four replicates per treatment using commercial formulations at highest recommended field concentration, half concentration and quarter concentration. The control block was treated with water. Assessments of the phsytoseiid were performed on mortality, escape of predators up to 5 days after the adult stage and reproduction of female during the first 5 days of the adult stage. Total effects (E) of the miticides were determined on the phytoseiid by combining lethal (mortality) and sub -lethal effects (fecundity). Persistence is classified by considering E suggesting that all fenpyroximate treatments and three days old residues of abamectin treatments would be the least compatible with *P. persimilis* but ten days old residues of abamectin treatments were favorable towards *P. persimilis.*

Key words: *Phytoseiulus persimilis*, persistence test, abamectin, fenpyroximate.

INTRODUCTION

Spider mites are one of the major pests of agricultural crops, especially in greenhouse, and traditionally controlled using pesticides sprays. Controlling greenhouse mite pests using chemical pesticides have resulted in problems such as the development of resistance in pests, increasing public concerns over the effect of pesticides on the environment and human health and also, increasing government regulations on pesticide use. Biological controls can overcome these problems while still providing adequate pest control (Helle and Sabelis, 1985; Garber et al., 1996) . 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 predaceous phytoseiid mite, *Phytoseiulus persimilis* Athias-Henriot is successfully used as biological control agent on two spotted spider mite, *Tetranychus urticae* Koch in a number of agricultural ecosystems (van Lenteren, 2000). The biological agent will not completely eliminate a mite pest problem, but will reduce mite pest populations and damage to an acceptable level. Thus, use of selective miticides is the first step in developing a mite management strategy within the framework of an integrated mite pest control program. In order to make

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Abbreviations: E, Total effects; **IPPRI,** Iran plant protection research Institute; **N,** highest field rate recommended; **IOBC,** international organization for biological control; **IMM,** integrated mite management; **IPM,** integrated pest management; **ANOVA,** analysis of variance.

Table 1. Miticides.

Active ingredient	Brand name	Field rate recommended (N) (ml/l)
Abamectin	Vertimec, EC 1.8%	0.2
Fenpyroximate	Ortus, SC 5%	0.5

use of the predaceous mite in the integrated mite management (IMM) program on crops, it is crucial to acquire information on the toxicity of commonly used miticides to the predator. No information is available on toxicities of the miticides to the predaceous mite *P. persimilis* on agricultural crops in Iran. Therefore, bioassay experiments were undertaken to assess the residual toxicity to *P. persimilis* of commonly two used miticides abamectin and fenpyroximate on *T. urticae*.

MATERIALS AND METHODS

Test principle

The *P. persimilis* protonymphs was exposed to aged residues of the test compounds on detached bean leaves. Commercial formulations of two miticides were tested for the persistence of their residual effects. The test was carried out according to the test method described by Oomen (1988) and Oomen et al. (1991).

Test organisms

The *T. urticae* and *P. persimilis* colonies originated from a natural infestation of plants in the greenhouse at Department of Agricultural Zoology, Iran plant protection research institute (IPPRI). The spider mites used as food for a stock colony of *P. persimilis* were maintained on bean plants (*Phaseolus vulgaris* L.) grown on plastic pots in growth chamber at 25 ± 2° , 65 ± 10% RH with 16:8 h (L:D) photoperiod.

The test method requires predator protonymphs of uniform age. Therefore a sufficient number of eggs (three times the number that was needed as protonymphs) was collected from the mass-rearing plants 3 days before the start of the test and were placed on detached bean leaf units for hatching at 25 ± 2° , 65 ± 10% RH with 16:8 h (L:D) photoperiod.

Application of test compounds

The *P. vulgaris* plants were treated with a commercial hand sprayer until spray deposit on the lower and upper side of the leaves were achieved. Test rates were N (highest field rate recommended), ½N and ¼N of miticides abamectin and fenpyroximate (Table 1). Bean plants, which were treated with tap water served as control samples.

Persistence tests on detached leaves

Three and ten days after the application, single bean leaves were detached with half petiole from the test plants and placed upside down on a water saturated cotton layer in a Petri dish (diameter 8) with a perforated base. Detached leaf edge was packed with cotton wool to create a 2 to 4 mm-high barrier around each leaf that extended to dish perimeter to prevent mites escaping from the leaf surface. The Petri dish was placed in water Petri dish (diameter 9) to provide a continuous water supply of the cotton layer. Then, predator protonymphs of uniform age were placed on the leaf surface 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. Mortality and escape of predators up to 5 days after the adult stage and reproduction per female during the first 5 days of the adult stage were assessed. All dead and live 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 from moment of first oviposition day during 5 days old period to assess the reproduction. All assessments were made with a stereomicroscope.

Analysis

Cumulative mortality was calculated by summing dead mites and dividing this number by the total number of live and dead mites at each mortality assessment, excluding unaccounted escapees (Blumel et al., 1993). The escape rate was calculated as a portion of 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 - Mortality corrected according to Abbott; M_t - Mortality in treatment; M_c - Mortality in control.

Possible changes in the number of females present on the test units during the reproduction period were taken into account by 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 - examples for evaluation adult day (d1: first adult day,…., d5; fifth adult day); R_{ry} - Reproduction in replicate number y; nE dx - number of eggs (in replicate number y) on day x; nF dx - number of females (in replicate number y) on day x.

Data on escape, mortality and female fecundity were analyzed using one-way analysis of variance (ANOVA) followed by the Duncan test to compare data means (SPSS, 2003). Effect on 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.

Subsequently effect on survival and 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; E - Total effect.

Based on total effects, rating of toxicity of miticides was evaluated according to international organization for biological control (IOBC) guideline (Blumel and Hausdorf, 2002).

Table 2. Percent escape of miticides after exposure to 3 and 10 days old residues of on *P. persimilis*.

Miticide	Concentration	Escape rate (Mean±SE)	
		3 days old residues	10 days old residues
Control	-	18.61 ± 1.77^{a}	24.16 ± 4.38^{ab}
Abamectin	N	20.00 ± 8.16^{a}	32.22 ± 3.33^{b}
Abamectin	½N	26.66 ± 8.16^{a}	32.22 ± 3.33^{b}
Abamectin	¼N	21.66 ± 1.66^{a}	26.66 ± 2.72^{b}
Fenpyroximate	N	15.55 ± 5.66^{a}	6.66^{a}
Fenpyroximate	½N	11.66 ± 3.19^{a}	16.66 ± 3.33^{a}
Fenpyroximate	¼N	6.66 ± 2.72^{a}	6.66^{a}

Means in the same column followed by different letter are significantly different (*P*< 0.01, Duncan test).

Table 3. Percent mortality, fecundity and total effects (E) of miticides after exposure to 3 days old residues on *P. persimilis*.

Miticide	Concentration	Mortality rates (Mean±SE)	Total eggs/female (Mean±SE)	Total effect (%)	IOBC class*
Control	-	10.18 ± 1.01^{a}	15.34 ± 0.4^{a}	-	-
Abamectin	N	73.35 ± 6.26^{cde}	2.57 ± 1.09^{d}	94.86	3
Abamectin	½ N	62.20 ± 5.46^{c}	3.53 ± 1.37^{d}	89.97	3
Abamectin	¼ N	34.16 ± 7.02^{b}	9.09 ± 1.76^{b}	55.35	2
Fenpyroximate	N	82.14 ± 0.94^{e}	4.66 ± 0.62^{dc}	88.15	3
Fenpyroximate	½ N	77.73 ± 4.00^{de}	8.01 ± 1.27^{bc}	87.10	3
Fenpyroximate	¼ N	66.38 ± 2.51^{cd}	9.00 ± 1.06^{b}	77.99	3

Means in the same column followed by different letter are significantly different (P< 0.01, Duncan test); *2: slightly harmful, 3: moderately harmful to *P. persimilis* in IPM programs.

RESULTS

Escape rates

The escape rates of phytoseiids for the three days old residues were not significantly different from control samples but they were significantly different for ten days old residues between treatments (Table 2).

Mortality and fecundity rates and total effects

Three days old residues

Compared with the control, abamectin and fenpyroximate at the 3 concentrations led to significantly greater mortality and lower fecundity rates. The mortality rate caused by ¼N abamectin was statistically lowest among all miticide treatments. Also, the mortality and fecundity rates caused by ¼N of fenpyroximate was statistically lower and greater than N of fenpyroximate, respectively. Total effect values for abamectin and fenpyroximate at 3 concentrations, ranged from 55.35 to 94.86% and 77.99 to 88.15%, respectively (Table 3).

Ten days old residues

Abamectin and fenpyroximate at 3 concentrations led to significantly greater mortality rates compared with the control. Also, significant differences were observed between abamectin at 3 concentrations and fenpyroximate at 3 concentrations. The fenpyroximate treatments led to significantly lower fecundity rates compared with the control, while the abamectin treatments caused no significant differences on fecundity rates. Total effect values for the abamectin treatments ranged from 10.05 to 26.46%, whereas fenpyroximate treatments ranged from 90.24 to 95.9% (Table 4).

DISCUSSION

Although the detached leaf method somewhat is influenced by escape rate, it is easier to access and monitor for studying side effects of pesticides on phytoseiids compared to other methods like closed cell technique which is very complicated in arrangement. Our results showed that none of miticides treatments had any repellency effects on predatory mite *P. persimilis* compared with control. On the other hand, data in Table 2 indicated that there were statistically differences in the number of *P. persimilis* that escaped from the leaf surface between fenpyroximate treatments and abamectin treatments after exposure to ten days old residues. One main reason for these results may be due to high percent mortality caused by fenpyroximate that

Table 4. Percent mortality, fecundity and total effects (E) of miticides after exposure to 10 days old residues on *P. persimilis*.

Miticide	Concentration	Mortality rates (Mean±SE)	Total eggs/female (Mean±SE)	Total effect (%)	IOBC class*
Control	-	9.92 ± 1.23a	15.93 ± 0.11a	-	-
Abamectin	N	29.52 ± 2.89b	14.36 ± 0.62a	26.46	1
Abamectin	½ N	21.81 ± 1.96b	15.63 ± 0.18a	11.12	1
Abamectin	¼ N	22.21 ± 5.98b	15.83 ± 0.06a	10.05	3
Fenpyroximate	N	80.72 ± 2.12c	3.16 ± 0.51b	95.9	3
Fenpyroximate	½ N	74.70 ± 4.04c	5.00 ± 1.89b	94.53	3
Fenpyroximate	¼ N	83.63 ± 3.64c	5.74 ± 1.75b	90.24	3

Means in the same column followed by different letter are significantly different ($P< 0.01$, Duncan test).*1, harmless; 3, moderately harmful to *P. persimilis* in IPM programs.

can cut down chance of escape in the predatory mite and conceal repellent effects (Stolz, 1994; Kavousi and Talebi, 2003). Observations also indicated that the predator protonymphs greatly paralyzed in a few hours time after placing it on leaf substrate treated with fenpyroximate at 3 concentrations. Thus, paralysis of predator protonymphs may be an additional reason for lower percent escapes at fenpyroximate treatments besides high percent mortality, since the paralyzed mites are less mobile.

The persistence of biological activity of different treatments on bean leaves under laboratory conditions, assessed by measuring total effect (E) resulting after three and ten days exposure to residues at 3 concentrations, indicated that fenpyroximate at 3 concentrations had fairly great effect on *P. persimilis* and would be considered moderately harmful by IOBC classification. The release of the phytoseiid into crop treated with these compounds could be carried out with caution. Such effects of fenpyroximate treatments to the predatory mite (ranged from 66.38 to 83.63%) is attributed to significant increase in mortality and low rate of fecundity after exposure to the miticide treatments (Tables 3 and 4). Blumel and Hausdorf (2002) reported that application of miticide fenpyroximate (Naja® 050 EC) on *P. persimilis* at three different concentrations based on PIEC in both three and ten residues, caused a total effect size reduction between 54.4 and 96.7% which allowed a classification as slightly or moderately harmful according to IOBC classification. Recently, workers also showed that application of fenpyroximate (Fujimite® 5 SC, 62.5 ppm) on strawberry leaf surface caused great effects (E= 100%) on *P. persimilis* and *Galendromus occidentalis* for at least 5 weeks after treatment (Irigaray et al., 2007). In contrast, the results were not consistent with the findings of van de Veire et al. (2001) who reported that application of miticide fenpyroximate (Naja® , 40 ppm AI) on *Amblyseius californicus* (McGregor) was short lived (E= 8.6%). Results and previous report of non-susceptibility of other species (van de Veire et al., 2001) indicated that phytoseiids vary in their susceptibility to fenpyroximate.

Consequently, phytoseiid species needed to be examined independently to determine their compatibility with fenpyroximate in integrated mite management programs. Abamectin is a commonly used miticide that has been evaluated for side effects on several important predatory mites. Present study indicated that ten days old residues of abamectin at 3 concentrations were more selective for *P. persimilis* than the other miticide treatments by IOBC classification (Table 4). Thanks to low mortality rates and non-significant effects on fecundity and subsequently highest reductions in the total effect size, ranging from 10.05 to 26.46% therefore, the use of abamectin at 3 concentrations which can be incorporated into existing integrated pest management (IPM) programs by *P. persimilis* ten days after application is been suggested for. Results were consistent with a study done by Irigaray et al. (2007) on *P. persimilis* and *G. occidentalis* which indicated that abamectin (Agrimec® 15 EC, 93.0 ppm) had little effect on adult female mortality, fecundity and fertility.

Moreover, most workers had reported that abamectin could be a favorably selective miticide towards phytoseiids in integrated mite management (IMM) (Zhang and Sanderson, 1990; Shipp et al., 2000; Ibrahim and Yee, 2000). Notwithstanding, the outcomes cited above should point out various factors including concentration of formulation and age of residues jointly with difference in species that can largely show a discrepancy in toxicity of abamectin in phytoseiids, as Zhang and Sanderson (1990), Ibrahim and Yee (2000) and Shipp et al. (2000) discussed. Results also showed that three days after abamectin application, decreasing concentration up to ¼N abamectin caused significant differences in lethal (mortality) and sub-lethal (fecundity) effects compared with N and ½N abamectin, resulting in reduction of total effect (E= 55.35%). The findings also indicated that application of older residue of abamectin (10 days old) along with decreasing concentration can greatly reduce toxic effects of abamectin miticide. Therefore, determining appropriate times and concentrations (particularly lower concentrations) of abamectin application can improve using predatory mite *P. persimilis* in IMM programs.

In general conclusion, study has demonstrated that releases of *P. persimilis* are not compatible with application of fenpyroximate at 3 concentrations up to ten days under laboratory conditions thus suggesting that supplementary tests required for determining favorable or unfavorable effects of fenpyroximate in integration with *P. persimilis* under greenhouse and field conditions. In contrast, abamectin at 3 concentrations are likely to be compatible with *P. persimilis* and the predatory mite releases could be made in as less as ten days following application of abamectin.

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