SIDE-EFFECTS OF THREE ACARICIDES ON THE PREDATORY MITE, *PHYTOSEIULUS PERSIMILIS* ATHIAS-HENRIOT (ACARI: PHYTOSEIIDAE) UNDER LABORATORY CONDITIONS

Ahmad Nadimi*, Karim Kamali*, Masoud Arbabi and Fateme Abdoli*****

* Department of Entomology, Tarbiat Modares University, Tehran, Iran. E-mail: ahmad_nadimi@yahoo.com
** Department of Agricultural Research Zoology, Iranian Plant Protection Research Institute.
*** Department of Biology, Guilan University, Rasht, Iran.

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ABSTRACT: The predatory mite *Phytoseiulus persimilis* Athias-Henriot is an economically important species in integrated mite pest management and biological control of spider mites in many countries throughout the world. For optimal biological mite management, it is important to know if acaricides have adverse undesirable effects on the predatory mites. The toxic effects of hexythiazox (Nisorun®, EC 10%), fenpyroximate (Ortus®, SC 5%) and abamectin (Vertimec®, EC 1.8%) on *P. persimilis* were evaluated. The acaricides were applied on detached bean leaves using a Potter Tower spray which deposited 2 mg spray solution per cm^2. Percent predator mortality was evaluated from the protonymph up to the adult stage including first five days of the oviposition period. The results showed that the total effect values of all concentrations of hexythiazox were below the lower threshold thus it could be considered a harmless acaricide to this predatory mite. In contrast, the total effect of all concentrations of fenpyroximate, and field, as well as, one half the field concentration of abamectin were found toxic to predatory mite and above upper threshold.

KEY WORDS: *Phytoseiulus persimilis*, Side effect, Acaricide, Predatory mite

INTRODUCTION

The two spotted spider mite, *Tetranycus urticae* (Koch), is one of the most important mite pest species with a wide range of host plants and world distribution (Bolland *et al.*, 1998). In Iran it is found on a number of outdoor and indoor agriculture crops (Arbabi et al., 1997). Many efforts have been undertaken to manage *T. urticae* problems in agricultural crops such as the application of new acaricides with the lower concentrations and *release* of predacious mites such as *Phytoseiulus persimlis* in glasshouses on cucumbers (Arbabi, 2007) and in fields of beans, cotton as well as soybeans *(*Daneshvar & Abaii, 1994). Among glasshouse pests recorded in the world, spider mites are known for their high fecundity, short life span and several generations per season. Under these circumstances spider mites are quickly selected for pesticide resistance pesticides (Helle & Sabelis, 1985). It has gained increasing attention by research scientists in many parts of the world. Selective pesticides that can be used to control pests without adversely affecting important natural enemies are urgently needed. Testing programme

represented by IOBC (International Organization for Biological Control), is not only meant to provide valuable information on the side effects of pesticides on beneficial organisms but it also gives the testing members an opportunity to improve testing techniques, compare results and exchange experience with colleagues in the Working Group (Hassan et al., 1991).

Mass rearing and releasing natural enemies mainly phytoseiid mites are one of the goals of biological control of these pests in indoor and outdoor conditions (McMurtry & Croft, 1997). Biological control of these pests is increasing because of the pressure on growers to find alternatives to chemical pesticides (van Lenteren, 2000). In the presence of chemical applications, biological control of spider mites may be achieved by the selective use of pesticides that are less toxic to natural enemies than to pest species (Zhang & Sanderson, 1990). Ruberson et al. (1998) suggested that selective pesticide were the most useful tool of integration of biological control agents into pest control programs.

A strain of *P. persimilis* was introduced into Iran from the Netherlands (Department of Entomology, Wageningen Agricultural University) in 1988 (Daneshvar, 1989) and it was effective in controlling spider mites under greenhouses and outdoor conditions (Daneshvar & Abaii, 1994). However, Biological control of spider mites using this predaceous mite is effective only against low population densities of the pest (Pralavorio et al., 1985). When the population densities are high an acaricide treatment is needed to reduce the pest population before release of beneficial mites (Malezieux et al., 1992).

The effects of pesticides on *T. urticae* are being widely studied and its resistance to new products is frequently monitored (Castagnoli et al., 2005). Failures of chemical control of T. urticae caused by resistance have been reported in several countries for compounds, such as Hexythiazox (Herron & Rophail, 1993), Fenpyroximate (Sato *et al*., 2004) and Abamectin (Beers et al., 1998). Although various aspect of pesticide effects on P. persimilis have been studied by many workers in the past (Samsøe-Petersen, 1983; Zhang & Sanderson, 1990; Oomen et al., 1991; Blumel et al., 1993; Hassan et al., 1994; Shipp et al., 2000; Blumel and Gross, 2001; Cloyd et al., 2006). Only Kavousi & Talebi (2003) investigated side-effects of heptenophos, malathion and pirimiphos-methyl on *P. persimilis* in Iran. Moreover, there is no information on the susceptibility of this introduced strain to other pesticides, especially acaricides.

In this study, we report the effects of abamectin, fenpyroximate and hexythiazox on *P. persimilis* used in biological control programs in glasshouses. The three acaricides are currently used for control of spider mites in Iran. The results will be used to develop IPM programs with *P. persimilis* in agricultural crops.

MATERIALS AND METHODS

1. Origin and rearing of mites

The *T. urticae* strain originated from the glasshouse of the Department of Agricultural Zoology, Iran Plant Protection Res. Institute (IPPRI) and was reared on beans (*Phaseolus vulgaris* L. var. Lordegan) sown in earthen pots in several months. *P. persimilis* strain originated from IPPRI that was reared on bean plants for 13 years without exposure to pesticides.

The two species were mass reared on bean leaves placed upside down on a layer of water-saturated cotton in a Petri dish and surrounded by wet cotton-wool to prevent the mites from escaping and, at the same time, provide water. Mite cultures were maintained in a controlled climate chamber at 25 ± 2 ºĊ, 65 ± 10% RH with 16:8 h (L:D) photoperiod.

2. Test Units Environment

The test unit consisted of a detached bean leaf placed lower side on a layer of water-saturated cotton in a Petri dish (80-mm diameter) with a hole drilled in the center. The Petri dish was placed in another lager Petri dish (90-mm diameter) to provide a continuous water supply to the cotton layer. Thus predatory mites were provided with drinking water and a barrier that impeded their escape. It is very important that all leaves are of the same quality in tests that are to be compared. Young, dark green, primary leaves were chosen that were roughly 5.5 cm wide at the widest part near the base (Samsøe-Petersen, 1983). The bean leaves were excised with their petioles intact and placed upside down onto wet cotton, the petioles were immediately embedded in moist cotton to extend the high quality of leaves and initiate the growth of roots (Bernard et al., 2004). Test units were kept in a controlled climate chambers.

3. Preparation of the predator

The test was done with the most susceptible life stage, i.e. protonymphs (larvae are too fragile to be used). Protonymphs of uniform age obtained according to the procedure described by Bakker et al. (1992).

4. Acaricides

The toxicity of abamectin (Vertimec®, EC 1.8%), fenpyroximate (Ortus®, SC 5%) and hexythiazox (Nisorun®, EC 10%) were evaluated at N, 1/2N and 1/4N where N represents the field rate recommended in Iran. Tap water was used in the controls (Table 1).

5. Spraying

The experiment was carried out using the detached leaf method according to Oomen (1988). Single detached leaves were sprayed at day 0 of the experiment on the lower side with a potter spray tower (Burchard Manufacturing, Uxbridge, United kingdom) was calibrated to achieve a

wet deposit of 2 mg cm^{-2}. The dry residue was used to test contact toxicity to juvenile predators. After the spray residue had dried, 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. 60 predator protonymphs (15 × 4 replicates) were used in each test unit. Finally, a plastic mesh was provided in the center of cover of the Petri dishes.

6. Assessment

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 3 to 7 d after spraying. All assessments were made with a stereomicroscope.

7. Analysis

To avoid overestimating mortality, 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):

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

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:

$$R_{ry} = (nE_{d3} / nF_{d3}) + [nE_{d4} / ((nF_{d3} + nF_{d4})/2)] + [nE_{d5} / ((nF_{d4} + nF_{d5})/2)] + [nE_{d6} / ((nF_{d5} + nF_{d6})/2)] + [nE_{d7} / ((nF_{d6} + nF_{d7})/2)]$$

d3 to d7: examples for evaluation days
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

Mean values of the escape rate, of the mortality rate and of the reproduction per female of the different treatments were analyzed statistically. Data were checked for normal distribution with Anderson-Darling test (Minitab 13) and analyzed by univariate variance analysis

(ANOVA, Duncan-test; SPSS 13.0 for windows). Data were transformed before analysis (square root).
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 & 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 acaricides was evaluated through the Working Group's joint pesticide testing programme in guideline IOBC (Bakker et al., 1992):

Class 1: E<30% (harmless)
Class 2: 30<E<80 (slightly harmful)
Class 3: 80<E<99 (moderately harmful)
Class 4: E>99% (harmful)

RESULTS

There was a significant difference in 7 d cumulative mortality effects of all three acaricides at all three concentrations on *P. persimilis* (Table 2). Mortality was highest after exposure to fenpyroximate at all concentrations and abamectin at field rate (100% mortality). Application at half and quarter of the field rate of abamectin resulted in 62.27 to 71.23% mortality (Table 2). In contrast, *P. persimilis* exposed to dry residues of all three concentrations of Hexythiazox suffered only 5.43 to 18.44% mortality.

Acaricides differed significantly in their effects on female fecundity (Table 2). The lowest reproductive performance was caused by fenpyroximate at all three concentrations and abamectin at field rate. Fenpyroximate caused a complete cessation of egg lay. Application at half and quarter the field rate of hexythiazox increased the reproduction performance on *P. persimilis* (Table 2).

All three acaricides had no repellent attributes (Table 4). The results of total effects (E) of the product applications are listed in Table 3. When the toxic effects of the acaricides are classified according to IOBC classification, all three concentrations of hexythiazox were harmless

(class1, E<30). At one quarter the field rate, abamectin was moderately harmful (class 3, 80<E<99) and half the field rate, abamectin and all three concentrations of fenpyroximate were harmful (class 4, E>99).

DISCUSSION

Among the 3 acaricides evaluated, only hexythiazox was harmless to *P. persimilis*. Fenpyroximate at the 3 concentrations evaluated and abamectin at the field and one half the field rates were harmful to *P. persimilis*. The use of these two compounds in the field would probably result in severe reduction of *P. persimilis*. Thus they are incompatible in IPM programs using this species. Our results are consistent with results reported for fenpyroximate and abamectin (Blumel & Hausdorf, 2002). Even at one quarter the field rate, Abamectin was moderately harmful to *P. persimilis*. Based on our observations these effects could be caused by a direct effect of these two acaricides on survival and reproduction of the predator mite.

Although various phytoseiid species have responded differently to abamectin, a reduction in reproduction is common to all (Zhang & Sanderson, 1990). Kim et al. (2005) showed that application of abamectin was highly toxic to *Amblyseius cucumeris* (Oudemans) adult females causing 92% mortality at 168 h after treatment and the number of eggs deposited by adult female predators decreased to 5.4 compared to 131.6 in the control.

Zhang and Sanderson (1990) believe that one reason of fewer egg produced is reducing mobility and thus consuming fewer prey. Also, they suggested that a lack of prey and quick elimination of spider mite by these acaricides may cause such effects.

Application of Hexythiazox at different concentrations was harmless to *P. persimils.* Our results are consistent with the results by Oomen et al. (1991), Hassan et al. (1987, 1991), van der Staay (1991) and Blumel & Gross (2001). It would be an appropriate substitute to fenpyroximate and abamectin in integrated pest management (IPM) programs.

Our observations showed that exposure to hexythiazox at one half and one quarter the field rates increased fecundity of *P. persimilis*. These results are not the first documented case of pesticide increasing fecundity in a phytoseiid mite. Kavousi & Talebi (2003) showed that heptenophos at the recommended concentration increased the fecundity of *P. persimilis*. Also, James (1997) reported increased fecundity in *Amblyseius victoriensis* by imidacloprid. The fecundity-enhancing property of hexythiazox can make *P. persimilis* an excellent choice as a biological control agent in greenhouses and other horticulture crops.

Van de Vrie et al. (1972) believed that certain pesticides can stimulate mite reproductive physiology; therefore, positive effect of hexythiazox at these two concentrations on reproduction may be physiological. Our results indicated that further studies on the effect of hexythiazox on fecundity and reproduction of *P. persimilis* and other phytoseiid species

are clearly warranted. For example, investigation of different concentrations of pesticides (especially lower rates) and comparative effects on the other stages should be assessed.

The relative toxicity of pesticides to pests, predators and immature stages (e.g. neonates) of the predators should provide an adequate indication for selectivity of pesticides, which is essential for development of pest management programs (Jeppson et al., 1975). Nevertheless, few populations consist of one life stage in nature and a true estimate of effect will not be gained by testing neonates only. If there is differential susceptibility among life stage, population toxicology is warranted (Stark & Banken, 1999). Furthermore, less susceptible stages can compensate for the loss of young and an accurate estimate of the toxic effect is therefore not obtained when toxicological studies are conducted with neonates only (Stark & Wennergren, 1995; Kareiva et al., 1996; Walthall & Stark, 1997; Stark et al., 1997). Ultimately, Stark & Banken (1999) suggested that to conduct more realistic toxicological studies, it is probably best to test a mixed age population.

Blumel et al. (2000) suggested that studies should be focused on the protonymph the most susceptible developmental stage, we suggest that side-effects of hexythiazox and other pesticides should be studied on other life stages.

There were no differences in the number of *P. persimilis* that escaped in treatments, but percentage was higher in control (25% escapes). The predatory mite, *P. persimilis* is a highly motile active predator, so higher escape levels are not surprising. Also, escaping from the treated test surface is a common problem in this method (Kavousi & Talebi, 2003). However, escape is a change in the behavior of the test mites, which as a test parameter should be addressed at higher test tiers (i.e. semi field and field trials) (Blumel et al., 2000).

It seems likely that several factors are affected on estimating the escape rate under laboratory conditions:

a) lethal effect of acaricides may conceal their repellent effects
b) handling of test units including adding food, removing eggs and dead mites and even light produced by stereomicroscope may cause overestimation in escape rates as repellent effects.

Thanks to the reasons cited above, as well as the high escape rates observed in the control block, it was not possible to estimate this parameter.

CONCLUSION

Of the three acaricides evaluated in the laboratory, hexythiazox may be incorporated in IPM programs based on *P. persimilis* without any additional studies. The other two acaricides fenpyroximate and abamectin were too toxic. A more detailed understanding of their toxicity under field conditions is required before any recommendations for their suitability or unsuitability in IPM programs in Iran can be made.

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Table 1. Acaricides

Active ingredient	Brand name	field rate recommended (N) (ml/l)
hexythiazox	Nisorun, EC 10%	2.5
abamectin	Vertimec, EC 1.8%	0.2
fenpyroximate	Ortus, SC 5%	0.5

Table 2. Effect of three acaricides at different concentrations on the survival and fecundity of *P. persimilis*

atments	Concentrations	% Mortality rates* (Mean±SE)	Total eggs/female* (Mean±SE)
ontrol	-	-	15.61 ± 0.33^{b}
ythiazox	N	18.44 ± 2.86^{a}	15.53 ± 0.27^{b}
ythiazox	1/2N	4.49 ± 3.19^{a}	19.12 ± 0.28^{a}
ythiazox	1/4N	5.43 ± 2.46^{a}	20.00 ± 0.78^{a}
mectin	N	100 ± 00^{c}	no surviving female
mectin	1/2N	71.23 ± 4.21^{b}	0.13 ± 0.47^{d}
mectin	1/4N	62.27 ± 3.33^{b}	3.01 ± 0.03^{c}
roximate	N	100 ± 00^{c}	no surviving female
roximate	1/2N	100 ± 00^{c}	no surviving female
roximate	1/4N	100 ± 00^{c}	no surviving female

*Means in columns followed by different letters are significantly different; Duncan-test; $P < 0.05$

Table 3. Total effect and toxicity of three acaricides at different concentrations on *P. persimilis* (IOBC evaluation categories).

Treatments	Concentrations	Total effects	Toxicity class
Control	-	-	-
hexythiazox	N	23.7	1
hexythiazox	1/2N	-15.29	1
hexythiazox	1/4N	-9.11	1
abamectin	N	100	4
abamectin	1/2N	99.73	4
abamectin	1/4N	92.24	3
fenpyroximate	N	100	4
fenpyroximate	1/2N	100	4
fenpyroximate	1/4N	100	4

Table 4. Repellency of *P. persimilis* after exposure to fresh residues of acaricides at different concentrations

Treatments	Concentrations	% Escape rates* (Mean±SE)
Control	-	25.00±94[a]
hexythiazox	N	21.66±0.83[a]
hexythiazox	1/2N	10.83±1.56[a]
hexythiazox	1/4N	20.00±3.33[a]
abamectin	N	15.00±0.83[a]
abamectin	1/2N	23.33±2.88[a]
abamectin	1/4N	21.66±0.83[a]
fenpyroximate	N	15.00±0.83[a]
fenpyroximate	1/2N	16.66±2.88[a]
fenpyroximate	1/4N	16.66±2.15[a]

*Means in columns followed by the same letter are not significantly different; Duncan-test; $P > 0.05$