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Effect of Frequent Application of Hexythiazox on Predatory Mite *Phytoseiulus persimilis* Athias - Henriot (Acari: Phytoseiidae)

[1]*Elham Sanatgar,* [1]*Reza Vafaei Shoushtari,* [2]*Abbas Ali Zamani,*
[3]*Masoud Arbabi and* [1]*Ebrahim Soleyman Nejadian*

[1]Department of Entomological, Agricultural Faculty, Islamic Azad University, Arak Branch, Arak, Iran
[2]Department of Plant Protection, Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran
[3]Department of Agricultural Zoology Research, Agricultural Acarology Laboratory, Iranian Research Institute of Plant Protection, Tehran, Iran

Abstract: Managing of *Tetranychus urticae* Koch, the economic pest of plants, is mainly based on the use of acaricides. The mite outbreaks are induced by a number of factors, particularly by the use of non-selective pesticides. Frequent application of these chemicals stimulates the mite resistance and suppresses the populations of natural enemies. *Phytoseiulus persimilis* Athias-Henriot is an important predator of *T. urticae*. Therefore, it is required to evaluate the effect of frequent application of acaricides on biological parameters of this predator. The acaricide hexythiazox (Nisorun®, EC 10%), was sprayed in 3 treatments of 0 (control=first generation), 7 and 15 times (generations) by a microner at the rate of 75 ppm a.i. on populations of the pest mite and its predator on the bean plants, *Phaseulus vulgaris*. The experiments were performed under laboratory conditions (27±2°C, RH: 65%±5 and L:D 16:8) on the bean leaves and the biological parameters of the predator were calculated after each frequency (number of generations) of the acaricide application. The values of r_m (Intrinsic rate of increase), λ (Finite rate of increase) and *DT* (Doubling time) were not different significantly for the 3 treatments, but R_0 (Net reproductive rate) and *T* (Mean generation time) were different. Duration of various stages among three treatments had significant difference but the total developmental time did not show any difference. Pre-oviposition period was longer after frequent spraying by hexythiazox. The overall conclusion shows that this acaricide can be used against *T. urticae* without inducing the adverse effect on population growth parameters of its predator, *P. persimilis*.

Key words: *Phytoseiulus persimilis* · *Tetranychus urticae* · Hexythiazox · Life Table · Predatory Mite · Two Spotted Spider Mite

INTRODUCTION

Predators belonging to the class Acari are important bio-control agents of plant feeder mites and they are one of significant parts of integrated pest management of these pests. Some of these Acari have been reported as a factor in regulating populations of phytophagus mites of economic importance, such as *Tetranychus urticae* Koch, *T. cinnabarinus* Boisduval, *Panonychus ulmi* Koch and *P. citri* McGregor [1].

The family Phytoseiidae from the class of Acari with about 2250 species has attracted considerable number of researches in the field of biological control of the mite pests [1-3]. Phytoseiid mites prey on a variety of food sources, however, some of them are host-specific [4]. For example, the four species of the genus *Phytoseiulus* feed only on spider mites [5]. The predatory mite *P. persimilis* Athias-Henriot (Phytoseiidae) is an effective biological agent of the spider mite *T. urticae* Koch (Tetranychidae) [6, 7].

P. persimilis was first described by Athias-Henriot in 1954. It is one of the most important predators of *T. cinnabarinus* and *T. urticae,* especially on protected crops [1, 2, 8-11]. This species is an obligatory predator of tetranychids and reared commercially on natural hosts, *Tetranychus* spp. using bean plants [1,12-14] and released widely throughout the world.

Corresponding Author: Elham Sanatgar, Department of Entomological, Agricultural Faculty, Islamic Azad University, Arak Branch, Arak, Iran.

T. urticae is an important, highly polyphagous and worldwide distributed pest of more than 900 host species [15] such as various species of orchard trees, field crops and greenhouses plants and induces serious damage by direct feeding [8, 16-19], reducing photosynthetic activity and leaf abscission in severe infestation [13] which describe this pest as a serious pest of at least 150 economically important plants [15]. In Iran, this pest is found on a number of outdoor and indoor agriculture crops [20]. Different control methods are used to manage this economic pest on agricultural crops. Application of acaricides in lower concentrations and release of predatory mite *P. persimilis* in glasshouses are two common methods to suppress the populations of *T. urticae* [21]. *T. urticae* outbreaks are induced by a number of factors, frequently by the use of non-selective pesticides towards decreasing its natural enemies and enhance pesticide resistance in populations [8, 22]. Since resistance to acaricides in *T. urticae* spread rapidly, biological control tactics are crucial to manage spider mite populations [8].

Studying the impact of common using pesticides on populations of *P. persimilis* is necessary to maximize the role of this bio-control agent [23]. Moreover, integration of *P. persimilis* and acaricides may be a powerful tool for the management of *T. urticae* populations and reducing the risks associated with the pesticide resistance inducing [23]. In the other words, integration of a biological control agent into agricultural IPM systems can not be achieved unless the natural enemy can survive the pesticides being used in that crop system [2].

To better estimate the side-effects of pesticides, there is an increasing attention and awareness to use more realistic endpoints. Demography has been suggested as the best way to combine lethal and sublethal effects and use to estimate the total effects of pesticides. Demographic toxicological analysis incorporates survivorship and reproduction of test organisms into one endpoint (i.e. population growth rate) [24].

Certain pesticides stimulate mite reproductive physiology [25]. Increasing fecundity induced by pesticides has been reported in several studies. For example, heptenophos at the recommended dose, increased the fecundity of *P. persimilis* [10] and imidacloprid increased fecundity of *Amblyseius victoriensis* (Womersley) [26]. Also, increasing fecundity of *P. persimilis* after exposure to hexythiazox has been reported [18].

Hexythiazox is a non-systemic acaricide with contact and stomach action from Thiazolidine group which has ovicidal, larvicidal and nymphicidal activity. It is not active against adults, but eggs laid by treated females are non-viable. This compound is used to control of many phytophagous mites (particularly *Panonychus*, *Tetranychus* and *Eotetranychus*) [27].

Some studies indicate that hexythiazox is harmless to *P. persimilis* [28] and used repeatedly in greenhouses. This research aims to study the effects of frequent application of hexythiazox on populations of *P. persimilis*.

MATERIALS AND METHODS

Origin and Rearing of Mites

Phytoseulus Persimilis: The strain of *P. persimilis* used in this study was provided from Koppert Company (www.koppert.com) and was reared on detached leaves of bean (*Phaseolus vulgaris* L. var. Akhtar) grown in pots for several months under condition of 28±2°C, RH: 20±5%, L:D 16:8. The leaves placed upside down on water-soaked cotton in 14×2cm petri dishes for at least 20 generations before initiating the experiments in germinator (27±2°C, RH: 65%±5 and L:D 16:8). The rearing units were checked every day to replace the old leaves with the fresh ones, adding enough prey and water. The number of mites which were needed for experiments was obtained from rearing arenas.

The experiments were conducted in 9.5×1cm petri dishes. One 20 mm hole on lid of each petri dish covered with a fine mesh fabric was provided for ventilation. The bottom of each petri dish was covered with water soaked cotton. The detached leaf of bean plant upside down was put on water-soaked cotton. In order to prevent mites escaping, a wet cotton-string surrounding the leaf area was provided.

Tetranychus Urticae: The strain of *T. urticae* used as the predator's prey in the experiments was originated from bean fields of Markazi province of Iran confirmed by Iranian acarologists. Then it was reared on bean in greenhouse under constant conditions of 28±2°C, RH: 20%±5 and L:D 16:8.

Pesticide: Hexythiazox (Nisorun®, EC 10%) was selected as an acaricide for experiments because of its widespread use on crops against two-spotted spider mites in greenhouses of the region. It was used at the same concentration recommended for field applications (75ppm a.i). The acaricide was diluted with distilled water to achieve the desired concentration. A model N14 Microner was used to cover evenly the whole spraying area.

Providing Cohort of Adult Predator: For the first experiment, 10 females of the predator mite were placed in each five 9.5×1cm petri dishes and removed them after 24 h. The cohort of eggs with 0-24 h old let to hatch and develop to adult stage. The leaves were replaced by fresh ones every three days if it was necessary. When most of adults emerged, the acaricide was applied for the first time and repeated every 5-7 days until 15 generations (15 times spraying). Petri dishes were checked every day to replace the old leaves with the fresh ones, adding enough prey and water to prevent escaping predators. The reproductive life table of the predator was provided for the control (first generation) and after seventh and fifteenth spraying.

Reproductive Life Table: In order to obtain a cohort, 30 mated females of predatory mite, *P. persimilis*, were placed in 6 petri dishes under conditions mentioned above. After 24 h, females were removed and each 0-24 h old egg was placed in a petri dish with the similar conditions (a detached bean leaf upside down on water soaked cotton and surrounding with cotton string) with enough prey (different stages of *T. urticae*). The predatory mite arenas were checked every 12 h for survivorship. When each female reached to the adult stage, a male was added to each arena. Couples were observed every day and the number of eggs laid per female/arena/day were counted and removed. Petri dishes were covered by parafilm to avoid escaping mites or entering mites from other places. The egg recording was being continued until the death of the last adult. The mites drown in wet cotton were disregarded from the experiment.

Statistical Analyses: A completely randomized design was used for all experiments. Data of developmental time and fecundity were analyzed using one-way ANOVA. Means were compared using Duncan's Multiple Range Test. To generating reproductive life table, daily age-specific survival (l_x) and fecundity rates (m_x) were used. The intrinsic rate of increase r_m was estimated by using the Lotka-Euler equation [29]: $\sum_{x=\alpha}^{B} e^{-r_m x} l_x m_x = 1$ where r_m is the intrinsic rate of natural increase, x is female age, l_x is the fraction of females surviving to age x and m_x is the expected number of daughters produced per female alive at age x, obtained by multiplying the number of eggs by the sex ratio. R_0 or net reproductive rate which is defined as the average number of female offspring that would be born to a birth cohort of females during their lifetime was calculated by summation of $l_x m_x$ ($R_0 = \sum_{\alpha}^{\beta} l_x m_x$). T (time required for a population to increase by a factor equal to the net reproductive rate), DT (the time required for the population to double) and λ (population increasing rate at each time step) calculated using the method recommended by Carey [30]:

(Mean `generation time) $T = \frac{In(R_o)}{r_m}$

(Doubling time) $DT = \frac{In(2)}{r_m}$

(Finite rate of increase) $\lambda = e^{r}$

RESULTS

Developmental Time and Survivorship: The predator mite successfully completed its development after all number of spraying (generations). Eggs passed through three immature stages including larva, protonymph and deutonymph. The developmental times of various stages of *P. persimilis* for the first generation (control) and under frequent application of hexythiazox for seventh and fifteenth generations are presented in Table 1.

Duration of various stages for control (first generation) and after 7 and 15 times spraying (7^{th} and 15^{th} generations) were significantly different but the total time (egg to adult) did not have significant difference (F=1.58; df=2,110; P=0.21). The predatory mite at the 7^{th} generation had longer embryonic period (F=25.84; df=2,113; P<0.0001) but larval and protonymphal stages were shorter (F=5.941; df=2, 111; P=0.004 and F=7.833; df=2, 110; P=0.001, respectively). Duration of deutonymph stage decreased in 7^{th} generation but increased after 15^{th} generation (F=29.96; df=2, 110; P<0.0001).

The age-specific survival (l_x) and age-specific fecundity (m_x) of *P. persimilis* reared under frequent application of hexythiazox for different generations are shown in Fig. 1. Survival curves were nearly similar in different generations and showed that most of mites completed their development to adult stage and preimaginal mortalities were not considerable. All mites died after 65, 34 and 45 days for the 1^{st}, 7^{th} and 15^{th} generations, respectively.

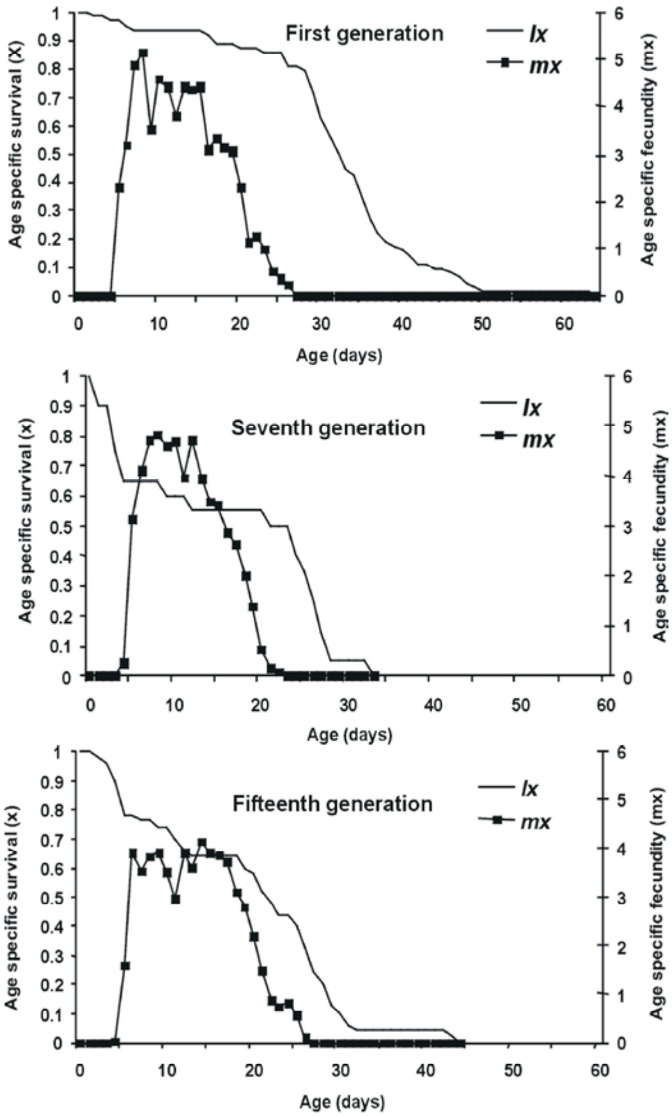

Fig. 1: Age-specific survivorship (lx) and age-specific fecundity (mx) of P. persimilis under frequent application of hexythiazox in the first, seventh and fifteenth generations

Table 1: Mean developmental times (days±SE) of immature stages of *P. persimilis* in the 1^{st}(control), 7^{th} and 15^{th} generations

Frequency of spraying (gen.)	Egg	Larva	Protonymph	Deutonymph	Egg to adult
0(1)*	1.18±0.04^{b**}	0.55±0.03^{a}	0.70±0.04^{a}	0.69±0.040^{b}	3.12±0.06^{a}
7	1.79±0.09^{a}	0.41±0.1^{b}	0.41±0.06^{b}	0.29±0.0615^{c}	2.91±0.2^{a}
15	1.31±0.05^{b}	0.39±0.03^{b}	0.56±0.05^{a}	0.92±0.05^{a}	3.15±0.06^{a}

* No spraying were applied in the first generation

**The means followed by different letters in the columns are significantly different

Table 2: Pre-oviposition, oviposition and post-oviposition periods (mean±SE) of *P. persimilis* in the 1^{st} (control), 7^{th} and 15^{th} generations

Frequency of spraying (gen.)	Pre-oviposition period (d)	Oviposition period (d)	post-oviposition period (d)
0(1)*	1.04±0.04^{b**}N=23	14.83±1.13^{a}	7.26±1.22^{a}
7	1.69±0.29^{ab} N=13	12.38±1.19^{a}	6.15±0.99^{ab}
15	2.26±0.31^{a} N=19	14.84±1.01^{a}	3.74±0.89^{b}

* No spraying were applied in the first generation

**The means followed by different letters in the columns are significantly different

Table 3: Effect of frequent applications of hexythiazox (0, 7 and 10 times) on the population growth parameters of *P. persimilis*

Parameter	1st generation*	7th generation	15th generation
Intrinsic rate of increase (r_m)	0.414±0.005^{a**}	0.404±0.020^{a}	0.380±0.011^{a}
Net reproductive rate (R_0)	58.50±2.85^{a}	37.78±5.63^{b}	43.60±1.94^{b}
Generation time (T)	9.83±0.12^{a}	9.04±0.20^{b}	9.94±0.26^{a}
Finite rate of increase (λ)	1.51±0.007^{a}	1.50±0.03^{a}	1.46±0.02^{a}
Doubling time (DT)	1.67±0.02^{a}	1.70±0.09^{a}	1.81±0.05^{a}

* No spraying were applied in the first generation

**The means followed by different letters in the rows are significantly different

Reproduction and Population Growth Parameters: The mites reared in the 1st (control) and 15th generation produced offspring for a longer time than mites reared in the seventh generation. However, these differences were not significant (Table 2). The rate of oviposition at the peak in the seventh generation was higher than two other generations.

Frequent application of hexythiazox exerted strong effect on pre-oviposition period (F=8.54; df=2,52; P=0.001), whereas, no significant differences were observed for oviposition (F=1.278; df=2,52; P=0.278) and post-oviposition period (F=2.914; df=2,52; P=0.63) (Table 2).

Growth Parameters: The estimated values of population growth parameters of *P. persimilis* under frequent spraying by hexythiazox during 1 (without spraying), 7 and 15 generations are represented in Table 3. The highest value of r_m was 0.414±0.005 for the first generation (control without any spraying). However, the effect of frequent application of hexythiazox was not significant on values of r_m after spraying in several generations (F=2.084; df=2,57; P=0.134). The values of net reproductive rate (R_0) were significantly different among various generations (F=9.416; df=2,57; P<0.0001). The R_0 was the highest in the first generation with 58.49±2.85 and the lowest in the seventh generation with 37.78±5.63 offspring per mite per generation. The highest mean generation time (T) was recorded for the 15th generation that was 9.94±0.26 and values of this parameter were significantly different among various generations (F=5.565; df=2,57; P=0.006). Other population growth parameters of *P. persimilis* such as finite rate of increase (λ) (F=2.153; df=2,57; P=0.126) and doubling time (DT) (F=1.649; df=2,57; P=0.183) were not significantly affected by frequent spraying by hexythiazox.

DISCUSSION

Studies on the development of predators are needed for the prediction of their population phenology and dynamics in field and greenhouse crops [31] and life table is a most important analytical tool, which provides detailed information of population dynamics to generate simple but more informative statistics. It also gives a comprehensive description of the survivorship, development and expectation of life [32].

Evaluating the toxicity of pesticides to natural enemies by measuring mortality alone underestimates the true effect of exposing to residuals [2]. In fact, many pesticides have little effect on mortality, but have considerable adverse effects on reproduction. Therefore, assessing the possible adverse effects of pesticides or acaricides on reproduction of natural enemies is also important in IPM programs. However, such methods of evaluating do not always allow an exact prediction of what happens in the field. Therefore, in this study, spraying method in the field was simulated in the laboratory and influence of frequent application of hexythiazox was assessed on development, mortality and reproduction of *P. persimilis*. Indeed, we knew that hexythiazox and *P. persimilis* alone has satisfactory performance against two-spotted spider mite. In this study we have investigated whether integrated use of both is possible to control *T. urticae*.

The obtained results showed that various frequent spraying of hexythiazox (1th, 7th and 15th generations) had no significant effect on total pre-imaginal developmental period of *P. persimilis* among various generations. However, comparison of each of the stages of larva, protonymph and deutonymph among different generations revealed that frequent use of hexythiazox caused a shorter development time for larvae and protonymphs. On the contrary, development time of deutonymph has increased after 15 generations or 15 times of spraying. With the knowledge that the highest feeding of immatures occurs during deutonymph stage, could be concluded that hexythiazox can even increase predation rate of *P. persimilis.* To verify this theory, more studies about foraging behavior and functional response of various life stages of *P. persimilis* is required. On the other hand, our results showed that frequent application of hexythiazox increased pre-oviposition period of *P. persimilis* after 7 and 15 generations. This is a negative characteristic in evaluation of a predator in biological control. It means that female oviposition starts later and consequently, the population growth rate of *P. persimilis* decreases when the oviposition period is constant. Time, is one of the most important factors in predator-prey population dynamics. Those predators who are able to increase their population in a shorter period, are the most capable candidate in biological control of their prey. Nevertheless, frequent spraying by hexythiazox has not changed significantly duration of oviposition period. Overall, adult longevity of *P. persimilis* is decreased after 7 and 15 generations (or spraying) and this means that feeding time of predator declined. In addition, the age-specific survival curve confirms this finding. As is shown in Fig. 1, mortality of all examined mites after 7 and 15 generations occurred in a shorter time. However, comparison of l_x curves for immature stages showed no clear differences among various generations. Therefore, we can conclude that hexythiazox has little effect on survival of immature stages and the most influence was occurred on adult mites.

Comparison of age-specific fecundity (m_x) curves (Fig. 1) showed that oviposition trend of female mites was considerably similar in the 7th and 15th generations and no clear peaks were observed during oviposition period. While, in the first generation (control without any spraying), a distinct peak was found on the 9th and 10th days of females' life and then oviposition rate gradually declined. In this situation, the most eggs laid at the beginning of oviposition period and this characteristic could increase ability of *P. persimilis* for increasing of its population in a shorter period.

Population growth parameters play a key role in evaluation of natural enemies and investigation on prey-predator population dynamics. In this regard, intrinsic rate of natural increase (r_m) considers as the most reliable parameter. Because various factors including age-specific survival (l_x), age-specific fecundity (m_x), sex ratio, developmental time and pre-imaginal mortality are involved for estimation of r_m. Based on the obtained results, frequent application of hexythiazox during several generations showed no significant effect on r_m-values. Thus, although there were significant differences for net reproductive rate (R_0) among various frequent generations, r_m is the most important factor for judgment.

Consequently, we can conclude that frequent spraying with hexythiazox has no negative impact on biological characteristics of *P. persimilis*. Increasing fecundity of *P. persimilis* after exposure to hexythiazox has been reported by Nadimi *et al* [18]. Also, increasing fecundity induced by other pesticides has been reported in several studies. For example, heptenophos at the recommended dose, increased the fecundity of *P. persimilis* [10] and imidacloprid increased fecundity of *Amblyseius victoriensis* (Womersley) [26]. These results are not consistent with our findings, because in this study net reproductive rate (R_0) decreased after frequent spraying with hexythiazox during several generations.

Duration of various stages of *P. persimilis* has been studied by various researchers at different conditions. At 25°C, egg, larva, protonymph, deutonymph and developmental stage lasted 34.83, 15.83, 24.11, 25.06 and 99.83 h, respectively [33] and 2.05; 0.45; 0.92; 1.04 (for egg, larva, protonymph and deutonymph respectively) in the same condition [34] that are not consistent with our observations. Davies and colleague [35] calculated duration of different stages of two strains (Tasmanian and New Zealand) of *P. persimilis* at 24°C. Comparison of their results and the results obtained in this research indicated that in both strains, the developmental time of different stages were higher than our calculations. The difference might be due to difference in temperatures of the two researches.

Hoque and colleagues recorded 0.1025-0.1823 for r_m value in different seasons at 25°C [36], whereas in the present study r_m was calculated 0.39-0.41. It is suggested that the difference is due to temperature. They also found the mean generation time of 10.31-20.83 days which was higher than ours for the same reason. Takafuji and Chant [37] recorded the value of T 13.1 days for *P. persimilis* at the condition of 25 ±1°C; 80±5% RH and 16:8 h (L:D) that is higher than our value for mean generation time.

Our results for r_m and λ is close to what Badii and McMurtry [38] recorded (0.374 and 1.453 respectively) and is comparable to results of Takafuji and Chant [37] that reported r_m =0.317, R_0= 63.2 and λ= 1.373. Abad-Moyano and colleagues [34] found the growth parameters of *P. persimilis* on clemantine. They calculated r_m=0.349 which is close to our results. However, their R_0 = 2.63 was lower than ours. Galazzi and Nicoli [7] calculated R_0 on different strains of *P. persimilis* during mass reproduction. They found a wide range of 30.1-46.7 for R_0 but their r_m was between 0.363 and 0.395 that was close to our observations.

The obtained results in this study revealed that frequent application of hexythiazox has no significant adverse influence on biology of *P. persimilis*. However, this alone is not enough on judgment of efficiency of integrated use of hexythiazox and *P. persimilis* to control the two-spotted spider mite. Indeed, more studies are required to understand the foraging behavior and predation rate of *P. persimilis* during different generations. In this regard, more studies should be done on functional response and mutual interference of *P. persimilis* under frequent spraying by hexythiazox.

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