The Sublethal Effects of Thiamethoxam, Hexaflumuron, and Acetamiprid Insecticides on the Growth Period, Reproduction, and Life Table of the Two-Spot Ladybirds (*Adalia bipunctata* Linnaeus, Col. Coccinellidae) Feeding on Common Pistachio Psylla

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Abstract

Introduction: The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Aphalaridae) is the key pest of pistachio trees in Iran. Controlling this pest is conducted by using chemical pesticides; this results in the destruction of the natural enemies of this pest and its outbreak. *Adalia bipunctata* Linnaeus (Col.: Coccinellidae) is one of the predators of pistachio psylla. Given the usage of three common pesticides i.e. thiamethoxam, hexaflumuron, and acetamiprid in pistachio gardens, the present study aims to investigate the sublethal effects of these three pesticides on the biological indictors of A. *bipunctata* (two-spot ladybirds) in controlled conditions.

Materials and Methods: In the present study, the effects of thiamethoxam, hexaflumuron, and acetamiprid pesticides have been investigated on some of the biological indicators and life table of this ladybird in controlled laboratory conditions (temperature of 27.5± 1 degrees centigrade, relative humidity of 65± 5 percent, and a photoperiod of 16 hours of light and 8 hours of darkness). This experiment has been conducted by immersing the ladybird's eggs in a toxic solution with sub-lethal concentrations. In order to investigate the sublethal effects of the insecticides, the growth period of immature and adult stages and the daily spawning rate of adult insects were investigated.

Results: The length of the pre-adult period in thiamethoxam, hexaflumuron, acetamiprid and control treatments were 16.69, 16.07, 16.34 and 15.58 (days) respectively. The insecticide treatments were significantly different from the control. The net fertility rate was higher in control and hexaflumuron treatments; they were significantly different from those of thiamethoxam and acetamiprid. The net reproduction rate (R_0) in thiamethoxam, hexaflumuron, acetamiprid and control treatments were respectively 41.87, 110.94, 62.2 and 153.7 (female/female/generation); a significant difference was observed between insecticides and control treatments. The highest intrinsic rate of population increase (r_m) was 0.151 (per day) in the control treatment; this was significantly different from that of the other treatments. The mean generation times (T) in thiamethoxam, hexaflumuron, acetamiprid and control treatments were respectively 42.98, 44.67, 38.12 and 33.01 (days); the treatments were significantly different.

Conclusion: The usage of these three pesticides in pistachio gardens reduces population growth indices and reproductive parameters of *A. bipunctata* ladybird. However, the side effects of hexaflumuron pesticide are less than the other two pesticides. This information can be used in the integrated control management of this pest.

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1. Introduction

Pistachio (*Pistacia vera* L.) is one of the most important garden products in Iran. According to the available evidence and sources, pistachio tree is attacked by a large number of pest arthropods (more than 50 species), especially insects [1, 2]. Common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hemiptera: Aphalaridae) is a key pest of pistachio trees in Iran. This insect feeds on the plant sap during the nymph and adult stages, and causes economic damage to pistachio trees [3]. This pest with a high reproductive power as well as numerous generations produce very high populations in one or more period in most years. The high densities of the insect population cause the general weakness of pistachio trees and the fall of leaves and buds, and bring about small fruits and increased percentage of fruit blankness and non-splitting. These trees are severely weakened and the general weakness of the tree and the loss of buds will also affect next year's crop [4- 6]. This pest produces 6 complete generations and one incomplete generation per year in Rafsanjan [7].

The common pistachio psylla has several natural enemies in pistachio-growing areas of Iran. Mehrnejad has reported more than 23 species of natural enemies for this pest [5, 6]. Several species of ladybirds attack the eggs and nymphs of the psylla [8, 9]. In this regard, 20 species of ladybirds on pistachio trees and 15 species on *Pistacia atlantica* subsp. *mutica* have been reported in pistachio orchards and wild pistachio habitats in Shahr-e Babak [10]. Two-spot ladybirds or *A. bipunctata* have also been collected on pistachio trees and weeds. This ladybird also prefers to lay eggs and live on pistachio trees to weeds infected with aphids. The common pistachio psylla eggs and nymphs are reported to be suitable prey for two-spotted ladybirds, and this insect is known to be an important predator of common pistachio psylla [6]. This insect has considerable psylla-eating and reproduction abilities [11, 12].

The evaluation of the effects of toxins on pests and their natural enemies should be comprehensive, taking into account the degree of lethality and physiological effects of toxins in sub-lethal concentrations. Therefore, it will not suffice to study the effects of toxins through conventional biometric methods in which only insect mortality is studied. Demographic toxicology method is a comprehensive research method in which not only the lethal effect of the toxin, but also their effects on life table parameters of an insect are investigated. This method has been recommended for evaluating the toxins especially the pesticides [13, 14].

Hexaflumuron insecticide is systemic and has a contact and digestive effect on

insects. This insecticide is one of the insect growth regulators (IGRs) and kills insects by preventing the formation of chitin and insects' molting. Acetamiprid is a neonicotinoid, systemic insecticide with contact and digestive effects that is used to control a wide range of pests of horticultural and agricultural products. The thiamethoxam insecticide belongs to the group of neonicotinoids. This systemic insecticide having contact and digestive function is used to control a wide range of sucking pests. In addition, these three insecticides are used in pistachio orchards to control pistachio psylla. The present study aims to investigate the effect of Hexaflumuron, thiamethoxam and acetamiprid insecticides on life table parameters and fertility of two-spot ladybird.

2. Materials and Methods

2.1- The conditions and the locations of experiments

The experiments were conducted in the laboratory of the plant protection department of Rafsanjan Pistachio Research Center. The experiments were performed in an incubator with a temperature of 27.5± 1°C, a relative humidity of 65± 5% and a photoperiod of 16:8.

2.2- Rearing and establishing a ladybird colony

The adult the two-spot ladybird was collected in May 2012 from pistachio trees of orchard located in the suburbs of Rafsanjan city and pistachio trees of station No. 2 of Pistachio Research Center. The insect was then transferred to the laboratory. Transparent plastic containers having the dimensions of 20 × 25 and a height of 10 cm were used for rearing adult insects. A hole with a diameter of 5 cm was made on the top of the containers, so that proper ventilation would be provided, and they were covered with a net cloth. In order to feed adult insects, leaves infected with normal pistachio psylla nymph were used. To prevent the growth of the fungus, the leaves in the growing containers were replaced on a daily basis. The leaves containing ladybird eggs were separated and transferred to petri dishes with a diameter of 6 cm. The petri dishes containing the eggs had a lid with a 1.5-cm diameter hole, and it was covered with a net. Given the cannibalism behavior of the larvae, the larvae were separately transferred to the petri dishes after being hatched. After rearing two generations by feeding on pistachio pyslla in the laboratory, the eggs of the third generation were used for conducting the main experiments [15].

2.3- pesticides

In the present study, thiamethoxam, hexaflumuron, acetamiprid pesticides have been applied. These pesticides have been introduced by Iran Plant Protection Organization for controlling different types of pests including common pistachio psylla (Table 1).

Table 1- Pesticides used in the experiments

General name	Trade name and producing company	Formulation	Sublethal concentration of the formulated substance
Hexaflumuron	Consult (DowagriSience)	EC 10%	400 ppm
Acetamiprid	Mospilan (Krishi, India)	SP 20%	50 ppm
Thiamethoxam	Actara (Syngenta)	WG 25%	200 ppm

2.4- Bioassay

For each treatment, 100 ladybird eggs being less than 24 hours old were selected. The eggs were immersed in the toxin solution with the leaves for 3 seconds. The treated eggs were transferred to petri dishes with a diameter of 6 cm and a height of 1 cm, and they were inspected on a daily basis. Moreover, the number of the hatched eggs and dead eggs were recorded. Blackened and non-hatched eggs were also recorded as dead eggs. After hatching, the larvae were transferred to separate petri dishes, and they were fed on a daily basis by using pistachio psylla nymphs until they became adult insects. The petri dishes of the larvae were inspected daily until the emergence of adult insects, and data related to the occurrence of each growth stage of the pest including the time of emergence of pupae, the emergence of adult male and female insects, and the rate of mortality at each growth stage were recorded. The sex of adult insects was determined as soon as they emerged. Male and female insects that appeared in one day were transferred to petri dishes to mate. Ordinary pistachio psylla nymph was used to feed the adult insects. Adults were inspected on a daily basis, and the number of eggs laid by each pair of ladybirds was counted and recorded. This procedure continued until the death of the last female insect [16].

2.5- Data analysis

Analyzing the data and calculating the reproductive parameters (such as gross fecundity rate, net fecundity rate, gross fertility rate, net fertility rate, etc.) and population growth parameters (such as gross reproductive rate, net reproductive rate, intrinsic rate of population increase, finite rate of population growth, mean generation time, doubling time, etc.) were performed based on Carey method (1993) and using Excel for each treatment separately. Pseudo-values were obtained by the Jackknife method. Statistical analysis was conducted by using SAS software Ver. 9.1 was done. The means obtained were then compared using Duncan's test at 5% level. Moreover, sustainable population parameters were calculated using the following equations:

The intrinsic rate of population increase

$$(r): \sum_{x=0}^{\omega} e^{-rx} l_x m_x = 1$$

The finite rate of population increase

$$(\lambda): \lambda = e^{r}$$

The intrinsic rate of birth (*b*):

$$b = \frac{1}{\sum_{x=0}^{\omega} e^{-rx} l_x}$$

The intrinsic rate of death (*d*): d= b-r

The gross reproductive rate: *GRR*=

$$\sum_{x=\alpha}^{\beta} m_x$$

The net reproductive rate (*NRR* or R_0):

$$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$$

The mean generation time (*T*):

$$T = \frac{\ln(R_0)}{r}$$

The doubling time (*DT*):

$$DT = \frac{\ln 2}{r}$$

3. Results

3.1- Biological parameters of the pest

The length of different growth stages of this ladybird in four treatments are shown in Table 2. The developmental period of the egg in three treatments of Thiamethoxam, Hexaflumuron and acetamiprid groups was longer than that of the control; this parameter was significantly different between the three treatments of insecticide and the control. The longest and shortest lengths of larval period were respectively observed in Hexaflumuron and control treatments; the larval periods were significantly different in the four treatments. Thiamethoxam pesticide increased the pupal length and was significantly different from those of the other three treatments. The shortest length of immature stage of this ladybird occurred in the control treatment; it was significantly different from those of the insecticide treatments (Table 2).

Table 2- The effect of insecticides on the length of growth stages of *A. bipunctata* ladybird by feeding on common pistachio psylla in laboratory conditions

Developmental stage	The length of the growth stage (days) in different treatment			
	Thiamethoxam	Hexaflumuron	Acetamiprid	Control
Egg	2.90 ± 0.04 a	2.988 ± 0.04 a	2.957 ± 0.04 a	2.7641 ± 0.03 a
Larva	9.533 ± 0.12 b	9.889 ± 0.15 a	9.056 ± 0.11 c	7.567 ± 0.06 d
Pupa	5.022 ± 0.06 a	4.856 ± 0.06 b	4.544 ± 0.06 c	4.367 ± 0.05 d
Immature stage	16.69 ± 0.18 a	16.07 ± 0.16 b	16.34 ± 0.17 ab	15.58 ± 0.10 c

Means within a row followed by different letters are significantly different

3.2- Growth and reproduction parameters

Reproductive parameters of this ladybird in four experimental treatments are given in Table 3. The lowest gross fertility rate was observed in Thiamethoxam treatment and was significantly different from that of the other treatments and control. The gross fecundity rates in Thiamethoxam, Hexaflumuron, Acetamiprid and control treatments are respectively 424.25, 566.72, 404.14, and 557.93 (eggs); Thiamethoxam and Acetamiprid were significantly different from the control. The highest net fertility and fecundity rates of this ladybird were observed in the control treatment which was significantly different from that of the other treatments (Table 3).

Table 3- Reproduction parameters of *A. bipunctata* ladybird in three insecticide treatments and control by feeding on common pistachio psylla at 27.5°C in laboratory conditions

Parameter	Treatments			
	Thiamethoxam	Hexaflumuron	Acetamiprid	water
Gross fertility rate	173.94 ± 0.317 d	357.03 ± 1.984 b	218.24 ± 2.388 c	485.39 ± 2.359 a
Gross fecundity rate	424.25 ± 2.680 b	566.72 ± 3.092 a	404.14 ± 4.422 c	557.93 ± 2.711 a
Gross hatching rate	0.41 ± 1.280 d	0.63 ± 5.360 b	0.54 ± 2.670 c	0.87 ± 6.590 a
Net fertility rate	38.07 ± 0.064 d	134.09 ± 0.744 b	65.57 ± 0.719 c	265.09 ± 1.155 a
Net fecundity rate	92.77 ± 0.155 d	212.85 ± 1.182 b	121.43 ± 1.332 c	304.71 ± 1.328 a

Means within a row followed by different letters are significantly different

The population increase parameters of this ladybird in three treatments and control are shown in Table 4. The net reproductive rates (R_0) for thiamethoxam, hexaflumuron, acetamiprid, and control were obtained to be 41.87, 110.94, 62.2, and 153.7 (female/female/generation) respectively; a significant difference was observed. The intrinsic population increase rate (r_m) for thiamethoxam, hexaflumuron, acetamiprid insecticides were respectively 0.087, 0.105 and 0.108 (per day); in comparison to the increase rate of the control i.e. 151.15, a significant difference has been observed. The highest finite rate of population increase was obtained in the control treatment with a value of 1.164; it was signficantly different from the value of this parameter in the treatments of thiamethoxam, hexaflumuron, and acetamiprid (Table 4).

Table 4- Values of population growth parameters of *A. bipunctata* ladybird on three insecticide treatments and one control treatment feeding on common pistachio psylla

Parameter	Treatments			
	Thiamethoxam	Hexaflumuron	Acetamiprid	Water
Gross reproductive rate (*GRR*) (female/female/generation)	190.9 ± 1.297 d	294.7 ± 1.698 a	206.1 ± 1.846 c	279.5 ± 1.623 c
Net reproductive rate (*NRR* or R_0 (fmeale/female/generation)	41.87 ± 0.243 d	110.94 ± 0.539 b	62.2 ± 0.718 c	153.7 ± 0.802 a
Intrinsic rate of population increase (r_m) (per day)	0.087 ± 0.002 c	0.105 ± 0.006 b	0.108 ± 0.004 b	0.151 ± 0.005 a
Intrinsic birth rate (*b*) (per day)	0.197 ± 0.0002 a	0.163 ± 0.0006 c	0.191 ± 0.0003 b	0.163 ± 0.0003 c
Intrinsic rate of death (*d*) (per day)	0.110 ± 0.00007 a	0.057 ± 0.00009 a	0.082 ± 0.00003 a	0.011 ± 0.00003 b
Finite rate of population increase (λ) (per day)	1.091 ± 0.0002 c	1.111 ± 0.0005 b	1.114 ± 0.0003 b	1.164 ± 0.0004 a
Mean generation time (*T*) (day)	42.98 ± 0.109 b	44.67 ± 0.105 a	38.12 ± 0.049 c	33.01 ± 0.005 d
Doubling time (*DT*) (day)	7.978 ± 0.015 a	6.576 ± 0.028 b	6.398 ± 0.015 c	4.562 ± 0.011 d

Means within a row followed by different letters are significantly different

The doubling times for thiamethoxam, hexaflumuron, and acetamiprid treatments were respectively 7.978, 6.576 and 6.398 (days); they were significantly different from the control treatment's value i.e. 4.564 (days). The lowest mean generation time (*T*) was observed in the control treatment, and it was significantly different from that of the insecticide treatments (Table 4). The results of analysis of variance and statistical calculation between different treatments as an independent factor and reproductive parameters and population increase as dependent variables indicate that between the variables of gross reproductive rate ($F= 1049.15$, $df= 3.44$, $P<0.01$), the net reproductive rate ($F= 6649.09$, $df= 3.44$, $P<0.01$), the intrinsic rate of population increase ($F= 40.95$, $df= 3.44$, $P<0.01$), the finite rate of population increase ($F= 548/985$, $df= 3.44$, $P<0.01$), the intrinsic birth rate ($F= 1382.5$, $df= 3.44$, $P<0.01$), and the doubling time ($F= 6207.105$, $df= 3.44$, $P<0.01$) there is a significant difference at the level of 1%.

4. Discussion

The results of present study indicated that the usage of pesticides reduces reproductive parameters such as gross fertility rate and net fertility rate and fecundity. Moreover, this indicated that the reduced spawning rate and reproductive parameters in Hexaflumuron is less than that of the other two insecticides. The studies conducted by Smith (1966) have showed that the sub-lethal concentration of malathion on the *sexmaculatus Menochilus* ladybird in a dripping method would result in the reduced number of eggs laid. In another study, imidacloprid and abamectin resulted in the reduced fertility, spawning rate, gross and net fertility rate, and egg hatching rate in *Criptolaemus monterozei* ladybird [17]. The results of these two studies are in line with those of the present study.

The intrinsic rate of population increase of this ladybird has reduced under the effect of sublethal dose of tested insecticides in comparison to the value of this parameter in the control group of the present study and other studies in which the insect has not been exposed to toxin; the aforementioned studies include those of Jalali [18] (0.18), Arabah Hormozabadi [19] (0.14), Atighi [20] (0.177) and Wahab-Zadeh [21] (0.172) have decreased. The finite population increase rates for the insecticides thiamethoxam, Hexaflumuron, and Acetamiprid were respectively 1.091, 1.111 and 1.114; it has decreased in comparison to the rate of the control group of this study (1.164), Arab-Hormozabadi [19] (1.15), and Wahhab-Zadeh [21] (1.187). The intrinsic population increase rate (r_m) is a good statistical basis for explaining the

population increase rate. The higher the value, the faster the population increase rate and the shorter the growth period will be [22]. However, it is worth noting that although r_m is an accurate parameter and can be used to compare the reproductive abilities of a given population, it is the influenced by many factors such as the species of the investigated insect, host type, geographical origin, climatic conditions (temperature, light, and humidity), the life span of adult insects, etc. [23]. Studies [24] have indicated that a natural enemy is suitable for biological control when its r_m is equal to or larger than the r_m of the related pest. Natural enemy species have lower population increase rates, longer life stages, longer developmental periods, and higher susceptibility to insecticides than their prey. Generally speaking, the population structure, when exposed to a toxic substance, plays an important role in the susceptibility of species having longer development time and lower population increase rate [13]. In the pest management programs, ecologist toxicologists put more emphasis on the use of low-persistence insecticides and insecticides having fewer side effects on natural enemies [25]. A study conducted by Ahmadi *et al.* (2009) indicated that imidacloprid and abamectin reduced the intrinsic rate of population increase in *Criptolaemus monterozei* Mulsant ladybird in comparison to the rate of the control treatment.In another study, the sublethal effect of three insecticides i.e. spirotetramat, fenitrothion and chlorpyrifos reduced population growth parameters in the *Oenopia conglobate contaminata* (L.) (Col. Coccinellidae) ladybird in comparison to the value of the control [26]. The results of these two studies are in line with those of the present study. As indicated in the present study, the gross and net reproductive rate, the intrinsic rate of population increase and the finite rate of population increase of Hexaflumuron treatment are higher than those of thiamethoxam and Acetamiprid. Thus, the side effects of Hexaflumuron insecticide are fewer than the other two insecticides. The usage of these three insecticides has resulted in the reduced intrinsic rate of population increase. Since the intrinsic rate of population increase is regarded as the most important feature and criterion for comparing the effect of a given treatment on an insect [27], it can be concluded that these insecticides leave adverse effects on ladybirds. As the rate of population growth decreases, its associated factors such as the doubling time and the finite rate of population increase are influenced as well. Since the doubling time (*DT*) of the population has increased in thiamethoxam, Hexaflumuron and Acetamiprid, the aforementioned insecticides result in the reduced efficiency of *A. bipunctata* ladybird in regulating the population of common pistachio psylla (host).

5. Conclusion

The results of the present study indicated that when it is not exposed to insecticides, predatory ladybird of *A. bipunctata* is in a favorable position in terms of ability and speed of population increase. However, the usage of three pesticides i.e. thiamethoxam, Hexaflumuron and Acetamiprid reduces the reproduction and speed increase of the population of this ladybird. However, this study shows that the reduction of spawning rate, reproductive parameters, and net reproductive rate in Hexaflumuron insecticide is less than thiamethoxam and Acetamiprid insecticides. Therefore, the sublethal effects of Hexaflumuron pesticide are less than the other two pesticides. These data can be used in the integrated control management of this pest.

Conflict of Interests

The authors of the present study confirm that there is no conflict of interests in publishing the present study; no remarkable financial support has been provided for the present study to influence its results.

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