

Lethal and sub-lethal effects of spirotetramat, thiamethoxam-lambda cyhalothrin and acetamiprid insecticides, on the biological parameters of *Oenopia conglobata contaminata* Menetries (Col.: Coccinellidae)

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Introduction: <i>Oenopia conglobata contaminata</i> Menetries is regarded as one of the most important natural enemies of pistachio psylla in the pistachio-growing regions of Iran. Given the usage of three insecticides i.e. spirotetramat, thiamethoxam-lambda cyhalothrin, and acetamiprid in pistachio orchards, the present study attempts to investigate the side effects of some insecticides on the biological parameters of <i>O. conglobata contaminata</i> in the laboratory conditions.</p> <p>Materials and Methods: In this study, the effects of 500 and 250 mg/l concentrations of Spirotetramat, 300 and 5 mg/l concentrations of thiamethoxam-lambda cyhalothrin, and 250 and 30 mg/l concentrations of acetamiprid, and distilled water as control, on life table and reproductive parameters of the ladybird was assayed. The experiments were performed by immersing of the ladybird eggs in the various treatments.</p> <p>Results: The maximum of egg development time was 3 days in related to the concentration of 5 mg/l thiamethoxam-lambda cyhalothrin. The highest larval period was observed in 250 mg/l concentration of acetamiprid. The adult longevity was the highest in the control and the lowest in the 30 mg/l concentration of acetamiprid. In concentration of 250 mg/l of acetamiprid, the longevity of the adult was significantly reduced compared to the control treatment. The lowest values of population parameters including r_m, R_0, GRR and λ were observed in acetamiprid treatment with a concentration of 250 mg/l. The results showed that the sides of effects in spirotetramat were less and safer than the other two insecticides.</p>
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1. Introduction

Pistachio (*P. vera*) is among the most significant orchard products having a high economic value and accounts for a high percentage of Iran's non-oil exports [1]. One of the major factors that reduce the pistachio yield in Iran's orchards is the activity of different kinds of pests. The common pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer (Hem.: Aphalaridae), is the economic pest of pistachio trees in Iran [2, 3, 4]. Moreover, this insect has been reported to be a key pistachio tree pest in the neighbouring countries including Armenia, Turkey, Iraq, as well as Mediterranean countries such as Syria and Greece [5, 6, 7, 8, and 9]. According to the studies conducted so far, pistachio psylla has several species of natural enemies; they can feed on this insect. They include parasitoid wasps as *Psyllaephagus pistaciae* Ferrière, ladybirds, green lacewings, predatory bugs and predatory mites. As many as eight species of ladybirds attack psylla in pistachio orchards; their psylla eating ability as well as their capability to grow and reproduce have been confirmed. From among them, *O. conglobata contaminata* is one of the most important predators of *A. pistaciae* in pistachio orchards [10, 11]. This ladybird ranks second and third in terms of abundance in pistachio orchards and wild pistachio (*Pistacia atlantica*) habitats, respectively. In recent years, controlling pistachio psylla by using chemical insecticides has eliminated the natural enemies of this pest. Thus, it is important to investigate the dimensions of the effect of chemical insecticides on the natural enemies. Demographic toxicology is a approach that has been proposed to evaluate different pesticides; in this method, in addition to the lethal effect, the

effects of toxins on the parameters of life table of an insect are examined as well [12]. Many insecticides are currently used to control of common pistachio psylla in orchards, including acetamiprid and spirotetramat. Chemical pesticides, such as thiamethoxam-lambda cyhalothrin are also used to control of other pistachios pests that can also have adverse effects on the natural enemies of psylla such as *O. conglobata contaminata*. Since the ladybird is one of the important factor in biological control of common pistachio psylla in IPM programs, it is thus required to conduct further studies on the biological effects of different insecticides on *O. conglobata contaminata*. Various researches have been conducted on the effect of some insecticides on the natural enemies of *A. pistaciae*. Some of these studies include "the lethal and sub-lethal effects of spirotetramat on the eggs of *Menochilus sexmaculatus* Fabricius [13], "Sublethal effects of three insecticides i.e. spirotetramat, Fenitrothion, and Chlorpyrifos on life parameters of *Oenopia conglobata contaminata*" [14], "the biological effects of acetamiprid and spirotetramat on *Hippodamia variegata* [15]. In this research, it has been attempted to investigate the lethal and sublethal effects of three pesticides i.e. spirotetramat, thiamethoxam-lambda cyhalothrin, and acetamiprid on the demography of *O. conglobata contaminata* in the laboratory conditions.

2. Materials and methods

2.1 Predator

In order to establish the initial colony of *O. conglobata contaminata*, the ladybirds were collected from pistachio-growing areas in the suburbs of Rafsanjan and moved to the laboratory. The ladybirds were collected from the pistachio orchards by shaking and jarring of branches of trees [16]. All stages of breeding and colony formation of this ladybird were conducted in vitro, inside a germinator with a temperature of 27.5 ± 1 ° C, $65 \pm 5\%$ RH, and 16: 8 L:D. Transparent plastic containers with dimensions of 20×25×10 cm were used for maintaining and breeding them. For providing proper ventilation, a hole with a diameter of 5 cm was made on top of the mentioned containers, and it was then covered with a mesh piece of cloth. In order to feed the adults and larvae, leaves infected with psylla nymphs that had not been sprayed, were collected and used in the garden on a daily basis. In order to prevent the growth of the fungi, the leaves in the breeding containers were daily replaced, and the leaves containing the ladybirds eggs were separated and transferred to the well-ventilated petri dishes. Since ladybird larvae have a cannibalistic behavior, the larvae were kept individually in 6-centimeter-diameter petri dishes after being hatched. The pups that appeared were kept under the same conditions until the emergence of adult insects. After the emergence of two generations of ladybirds fed with pistachio psylla, the eggs laid by the third generation of ladybugs were used for conducting the required experiments.

2.2 feeding

Nymphs of *A. pistaciae* were used to feed the larvae and adult of *O. conglobata contaminata* in the laboratory. Leaves containing psylla nymphs were daily collected from the pest-

infested orchard that had not been previously sprayed. The leaves were then moved to the laboratory and were given to the larvae and adult of *O. conglobata contaminata*.

2.3 Insecticides

In the present study, the three systemic insecticides include spirotetramat (Movento® 10% SC) (from the ketonol group and tetramic acid class), thiamethoxam-lambda cyhalothrin (Eforia® 247% SC), (combination of thiamethoxam from neonicotinoids and lambda cyhalothrin from pyrethroids), and acetamiprid (Mospilan® 20% SP) (from the neonicotinoids group) were used.

Since the recommended concentrations are normally used for pest control in pistachio orchards, these concentrations were used as the lethal concentrations for conducting the experiments. To determine the sublethal concentration of the insecticides, based on the recommended concentration, bioassay experiments were conducted with different concentrations. The concentrations by which less than 25% of mortality occurred for *O. conglobata contaminata* were determined as the sublethal concentration. The concentrations used for the present study include concentrations of 500 and 250 mg/l for spirotetramat, 125, 250, 62 and 30 mg/l for acetamiprid, and 300, 150, 75, 37, 18, 9 and 5 mg/l for thiamethoxam-lambda cyhalothrin.

2.4 Effects of insecticides on the demography of *O. conglobata conglobate*

The life table of the *O. conglobata contaminata* began with 150 eggs treated with spirotetramat, thiamethoxam-lambda

cyhalothrin, acetamiprid, and distilled water. These experiments were conducted at a temperature of 27 ± 1 ° C, a relative humidity of $65 \pm 5\%$, and 16: 8 L:D. The eggs treated with the insecticides were visited every 24 hours. The interval between oviposition time and hatching of the eggs was recorded as the period of egg incubation. By determining the ratio of the hatched and non-hatched eggs, the percentage of egg stage mortality was measured. In the larval period, their growth process was daily studied and the larval and pupae mortality rates were recorded daily. After the pupal stage, a cohort of adults, which emerging in one day, were transferred to the new petri dishes and the infected pistachio leaves were provided for them. The adults were visited daily and the number of eggs laid by each female was counted and recorded. In case of the death of male ladybird in each petri dish, one male from the rearing colony was replaced and this procedure was conducted until the death of the last female.

3. Data analysis

Data related to the effect of insecticides on the ladybird biostatistics were analyzed by using by of age-stage, two-sex life table method and Two Sex-Ms Chart software. Standard error of life table parameters was estimated by using the Bootstrap (n=10000) method (17, 18). It was attempted to calculate age-specific survival rate (l_x), age-specific fecundity (mx), age-stage specific fecundity (fx_j), age-stage specific survival rate (Sx_j), age-stage specific life expectancy (ex_j), age-stage specific reproductive value (vx_j); x and j stand for age and biological stage, respectively. Intrinsic rate of increase (r), finite capacity for increase (λ), net reproduction

rate (R_0), mean generation time (T) and gross reproduction rate (GRR) were calculated as well. In addition, the mean was compared with Duncan's Test at 5% level, and the graphs were drawn by using Sigma Plot V. 13.0 (Systat software Inc.).

4. Results

Effect of the insecticides on mortality rate of *O. conglobata* contaminate eggs

The mortality percentage of the tested insecticides on *O. conglobata contaminata* eggs is shown in Table 1. The effect percentage of each insecticide concentration was calculated using the Schneider-Orelli formula. The results showed that the mortality percentages for the spirotetramat at concentrations of 500 and 250 mg/l were 30 and 18.66 and the effective percentages was 18.39 and 16.05, respectively. The concentration of 250 mg/l was determined as the sublethal concentration. Moreover, the mortality percentages of acetamiprid at concentrations of 250 and 30 mg/l were 64.88 and 16.22 and the percentages of effect were calculated to be 59.05 and 14.31, respectively. In this insecticide, the concentration of 30 mg/l was determined as the sublethal concentration. Finally, the mortality percentages of thiamethoxam-lambda cyhalothrin at 300 and 5 mg/l concentrations were 100 and 24 and the percentages of effects were determined to be 100 and 18.83, respectively. The sub-lethal concentration for this insecticide was measured to be 5 mg/l.

Table 1. The effect of the insecticide on the mortality rate of *O. conglobata* contaminate eggs

thiamethoxam-lambda cyhalothrin			spirotetramat			acetamiprid			control (distilled water)
Concentration (mg/l)	Mortality percentage	Percentage of effect	Concentration (mg/l)	Mortality percentage	Percentage of effect	Concentration (mg/l)	Mortality percentage	Percentage of effect	Mortality percentage
300	100	100	500	30	18.39	250	64.88	59.05	14.22
150	100	100	250	18.66	16.05	125	52.88	51.37	3.10
75	100	100	-	-		62	36.22	31.17	7.33
37	100	100	-	-		30	16.22	14.31	2.22
18	88.66	87.84	-	-		-	-	-	6.71
9	62.55	59.85	-	-		-	-	-	6.71
5	24	18.83	-	-		-	-	-	6.71

Effects of the insecticides on developmental period of *O. conglobata contaminata*

The effect of the different concentrations of spirotetramat, thiamethoxam-lambda cyhalothrin and acetamiprid on immature developmental period, pre-adult period, adult period of *O. conglobata contaminata* has been shown in Table 2.

The results indicate that the spirotetramat and thiamethoxam-lambda cyhalothrin significantly affect the incubation ($df = 5$, $P < 0.0001$, $F = 2704.89$). The most significant effects were related to the thiamethoxam-lambda cyhalothrin with the concentration of 5 mg/l (3 days), spirotetramat with the concentration of 250 mg/l (2.35 days) and 500 mg/l (2.34 days) and these increased the incubation period of *O. conglobata contaminata*. The results indicate the effect of the insecticides on the first instar larval stage ($df = 5$, $F = 3997.65$, $P < 0.0001$). The most effective treatments in increasing of the first instar larval period were acetamiprid 250 mg/l (2.95 days). The shortest second instar larval period was observed in spirotetramat 500 mg/l. The highest third instar larval period was observed in acetamiprid 250 mg/l which it was significantly different from the other treatments ($df = 5$, $F = 2889.83$, $P < 0.0001$). The fourth instar larval period of the indicated the highest increase in

spirotetramat 250 mg/l and acetamiprid 250 mg/l in comparison to the control ($df = 5$, $F = 2965.02$, $P < 0.0001$).

The results indicate that the larval period in acetamiprid 250 mg/l, spirotetramat 250 mg/l, acetamiprid 30 mg/l, and thiamethoxam-lambda cyhalothrin 5 mg/l increased respectively in comparison to the control ($df = 5$, $F = 77.13$, $P < 0.0001$). The results also indicated that the pupal period of the ladybird was significantly affected by different concentrations of the insecticide; the most significant effect was observed in acetamiprid ($df = 5$, $F = 115.89$, $P < 0.0001$).

The pre-adult stages significantly increased in all treatments in comparison to the control ($df = 5$, $F = 4072.46$, $P < 0.0001$); the highest and lowest increase in the pre-adult period was respectively observed in acetamiprid 250 mg/l (20.29 days) and spirotetramat 500 mg/l (16.98 days). This indicates the less adverse effects of spirotetramat on *O. conglobata contaminata*. The results on the effect of the insecticides on the adult life span, show a significant reduction in the longevity of adult in the different concentration of treatments compared to the control ($df = 5$, $F = 6686.63$, $P < 0.0001$) and the highest decrease was recorded for acetamiprid and thiamethoxam-lambda cyhalothrin.

Table 2. The effect of the tested insecticides on the on different developmental periods of *O. conglobata contaminata*

Stage period (day)	spirotetramat	spirotetramat	thiamethoxam-	acetamiprid	acetamiprid	Control
	500 mg/l	250 mg/l	lambda cyhalothrin 5 mg/l	250 mg/l	30 mg/l	
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Egg	2.34 ± 0.004 ^c	2.35 ± 0.003 ^b	3 ± 0 ^a	2 ± 0 ^d	2 ± 0 ^d	2 ± 0 ^d
First instar	1.92 ± 0.007 ^d	1.72 ± 0.005 ^e	2.70 ± 0.01 ^b	2.95 ± 0.01 ^a	2.27 ± 0.06 ^c	1.67 ± 0.004 ^f
Second instar	2.07 ± 0.009 ^e	2.65 ± 0.01 ^b	2.29 ± 0.006 ^d	2.61 ± 0.12 ^c	2.61 ± 0.009 ^c	2.73 ± 0.007 ^a
Third instar	2.29 ± 0.009 ^e	3.40 ± 0.009 ^b	2.24 ± 0.007 ^f	3.47 ± 0.01 ^a	3.12 ± 0.009 ^c	3.02 ± 0.007 ^d
Fourth instar	4.78 ± 0.015 ^c	5.55 ± 0.015 ^a	4.46 ± 0.01 ^d	5.44 ± 0.02 ^b	4.41 ± 0.006 ^e	3.37 ± 0.008 ^f
Total larval period	11.14 ± 0.206 ^e	13.35 ± 0.012 ^b	11.60 ± 0.13 ^d	14.58 ± 0.26 ^a	12.32 ± 0.10 ^c	10.75 ± 0.109 ^e
Pupa	3.35 ± 0.023 ^e	3.53 ± 0.007 ^b	3.47 ± 0.007 ^c	3.67 ± 0.01 ^a	3.66 ± 0.006 ^a	3.41 ± 0.007 ^d
Total pre-adult	16.98 ± 0.013 ^e	19.24 ± 0.01 ^b	18.06 ± 0.01 ^c	20.29 ± 0.04 ^a	17.99 ± 0.014 ^d	16.16 ± 0.03 ^f
Adult	54.74 ± 0.27 ^b	49.45 ± 0.13 ^c	34.06 ± 0.10 ^d	30.80 ± 0.14 ^e	30.02 ± 0.06 ^f	61.17 ± 0.16 ^a
Male age	53.52 ± 0.41 ^b	49.76 ± 0.23 ^c	33.92 ± 0.27 ^d	29.01 ± 0.46 ^e	29.13 ± 0.19 ^e	57.17 ± 0.51 ^a
female age	56.33 ± 0.87 ^b	48.46 ± 0.27 ^c	33.19 ± 0.12 ^d	31.53 ± 0.21 ^e	30.50 ± 0.10 ^e	63.92 ± 0.23 ^a
Adult longevity	33.58 ± 0.22 ^c	34.42 ± 0.23 ^b	25.32 ± 0.17 ^d	20.04 ± 0.14 ^f	23.05 ± 0.14 ^c	38.63 ± 0.21 ^a
Male longevity	70.93 ± 0.38 ^b	69.055 ± 0.23 ^c	51.78 ± 0.27 ^d	49.11 ± 0.45 ^e	47.13 ± 0.19 ^f	73.43 ± 0.51 ^a
female longevity	72.73 ± 0.84 ^b	67.50 ± 0.27 ^c	52.12 ± 0.12 ^d	51.58 ± 0.20 ^d	48.49 ± 0.11 ^e	79.98 ± 0.24 ^a

Same letters in each row are not significantly difference (Duncan's Multiple-Range Test, p = 0.05)

Effects of the insecticides on the population parameters of *O. conglobata contaminata*

Table 3 indicates the population parameters including intrinsic rate of natural increase (r_m), net reproduction rate (R_0), gross reproduction rate (GRR), mean generation time (T) and finite capacity for increase (λ) of *O. conglobata contaminata* in the different treatments. The results showed that the intrinsic rate of natural increase was a significantly difference within of the different treatments (df= 5, F = 1031.81, P < 0.0001) and the lowest and the highest intrinsic rate of natural increase was respectively related to the acetamiprid 30 mg/l (0.06 days⁻¹) and thiamethoxam-

lambda cyhalothrin 5 mg/l (0.10 dya⁻¹). Moreover, the lowest finite capacity for increase was observed in the acetamiprid 30 mg/l (0.10 day⁻¹). The largest decrease in gross reproductive rate was related to acetamiprid 30 mg/l (41.08 offspring/individual).The net reproduction rate in all treatments was significantly reduced compared to the control (df = 5, F=905.38, P<0.0001) and the highest decrease was observed in acetamiprid 250 mg/l (9.32 offspring/individual). The highest mean generation time was reported for spirotetramat 250 mg/l (38.94 day) and 500 mg/l (37.65 day). Moreover, the lowest mean generation time was observed in acetamiprid 30 mg/l (31.21 day) and 250 mg/l (34.16 day).

Table 3. The effect of the tested insecticides on the population parameters of *O. conglobate*

Parameters	spirotetramat 500 mg/l	spirotetramat 250 mg/l	thiamethoxam- lambda cyhalothrin 5 mg/l	acetamiprid 250 mg/l	acetamiprid 30 mg/l	control
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
intrinsic rate of natural increase (r_m) (day⁻¹)	0.07 ± 0.0005 ^d	0.07 ± 0.0005 ^d	0.10 ± 0.0004 ^a	0.06± 0.0003 ^f	0.09 ± 0.0004 ^c	0.09 ± 0.0004 ^b
finite capacity for increase (λ) (day⁻¹)	1.08 ± 0.0005 ^d	1.07 ± 0.0005 ^e	1.10 ± 0.0004 ^a	1.06 ± 0.0003 ^f	1.09 ± 0.0004 ^c	1.10 ± 0.0004 ^b
gross reproduction rate (GRR) (offspring/individual)	63.14 ± 0.85 ^c	43.95 ± 0.68 ^e	75.34 ± 1.15 ^b	41.08 ± 0.45 ^f	48.44 ± 0.76 ^d	91.77 ± 0.71 ^a
net reproduction rate (R_0) (offspring/individual)	19.19 ± 0.33 ^c	18.91 ± 0.32 ^c	27.06 ± 0.35 ^b	9.32 ± 0.11 ^d	18.55 ± 0.22 ^c	36.44 ± 0.38 ^a
mean generation time (T) (day)	37.65 ± 0.11 ^b	38.94 ± 0.05 ^a	32.81 ± 0.04 ^e	34.16 ± 0.20 ^d	31.21 ± 0.02 ^f	36.40 ± 0.07 ^c

Same letters in each row are not significantly difference (Duncan’s Multiple-Range Test, p = 0.05).

Effects of insecticides on reproductive capacity of *O. conglobata contaminata*

The effect of the treatments on the reproductive capacity of *O. conglobata contaminata* is shown in Table 4. The results indicate that different treatments had significant effects on fecundity rate, adult pre-oviposition period (APOP), total pre-oviposition period (TPOP), and oviposition period of the ladybird.

The results indicated that fecundity of the female reduced significantly in thiamethoxam-lambda cyhalothrin 5 mg/l in comparison to the control (df=5, F=526.97, P<0.0001). The highest adult pre-oviposition period (APOP) was observed in spirotetramat 500 mg/l (8.11 day) that it was significantly different from the control (df=5, F=163.18, P<0.0001). The highest pre-oviposition period (TPOP) of the female was seen in spirotetramat 500 mg/l (24.83 day) and the shortest oviposition period was seen in spirotetramat 500 mg/l (12.50 day).

Table 4. The effect of the insecticides on the reproductive capacity of *O. conglobata*

Parameter	spirotetramat 500 mg/l	spirotetramat 250 mg/l	thiamethoxam- lambda cyhalothrin 5 mg/l	acetamiprid 250 mg/l	acetamiprid 30 mg/l	control
reproductive capacity	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Fecundity	96.99 ± 0.94 ^d	132 ± 1.42 ^a	107.46 ± 0.91 ^c	40.93 ± 0.40 ^f	76.46 ± 0.49 ^e	128.51 ± 0.78 ^b
adult pre-oviposition period (APOP) (day)	8.11 ± 0.16 ^a	3.99 ± 0.05 ^b	4.44 ± 0.03 ^b	3.23 ± 0.04 ^c	3.26 ± 0.02 ^c	3.79 ± 0.01 ^{ab}
total pre- oviposition period (TPOP) (day)	24.83 ± 0.38 ^a	23.03 ± 0.09 ^c	22.65 ± 0.03 ^c	23.55 ± 0.06 ^b	21.26 ± 0.03 ^d	19.85 ± 0.03 ^e
Oviposition period (day)	12.50 ± 0.28 ^c	19.95 ± 0.22 ^a	11.86 ± 0.07 ^d	8.78 ± 0.06 ^e	11.83 ± 0.06 ^d	17.04 ± 0.06 ^b
Pre adult survival rate	0.42 ± 0.003 ^b	0.46 ± 0.003 ^a	0.42 ± 0.003 ^b	0.32 ± 0.002 ^c	0.42 ± 0.003 ^b	0.46 ± 0.002 ^a

Same letters in each row are not significantly difference (Duncan’s Multiple-Range Test, p = 0.05).

Effect of the insecticides on age-stage life table *O. conglobata contaminata*

The age-stage specific survival rate (S_{xj}) indicates the probability of survival of newborn insects at age x and developmental stage j . The age-stage specific survival rate curve of *O. conglobata contaminata* treated with different insecticides can be seen in figure 1. The results indicate that this parameter in the female treated with spirotetramat 250 mg/l was lower than that of the male. Moreover, the value of this parameter in thiamethoxam-lambda cyhalothrin 5 mg/l, acetamiprid 250 mg/l, acetamiprid 30 mg/l, and spirotetramat 250 mg/l have considerably decreased.

Effects of the insecticides on age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *O. conglobata contaminata* are shown in Figure 2. In l_x diagram, mortality rate increased in all treatments in the early and late larval instars. Moreover, age-specific fecundity of the female (m_x) decreased in thiamethoxam-lambda

cyhalothrin 5 mg/l and Spirotetramat 250 mg/l and acetamiprid 30 mg/l in comparison to the control.

Age-stage specific reproductive value (V_{xj}) was first introduced by Fisher in 1930 [19] and refers to the number of offspring that expected produce by an individual at age x and stage j . This parameter expresses the relative participation of each age group in helping to the future generations [19, 20] and is shown in figure 3. The females had the largest effect on the reproduction value in the next generation. Except in Spirotetramat 500 mg/l, in all treatments, the reproductive value was higher than the control.

Age-stage specific life expectancy (e_{xj}) refers to the total duration individual life span which is expected to live at age x and stage j and generally, life expectancy decreases as an individual grows older. The effect of insecticides on the ladybird's life expectancy (figure 4) indicates that this reduced in comparison the control.

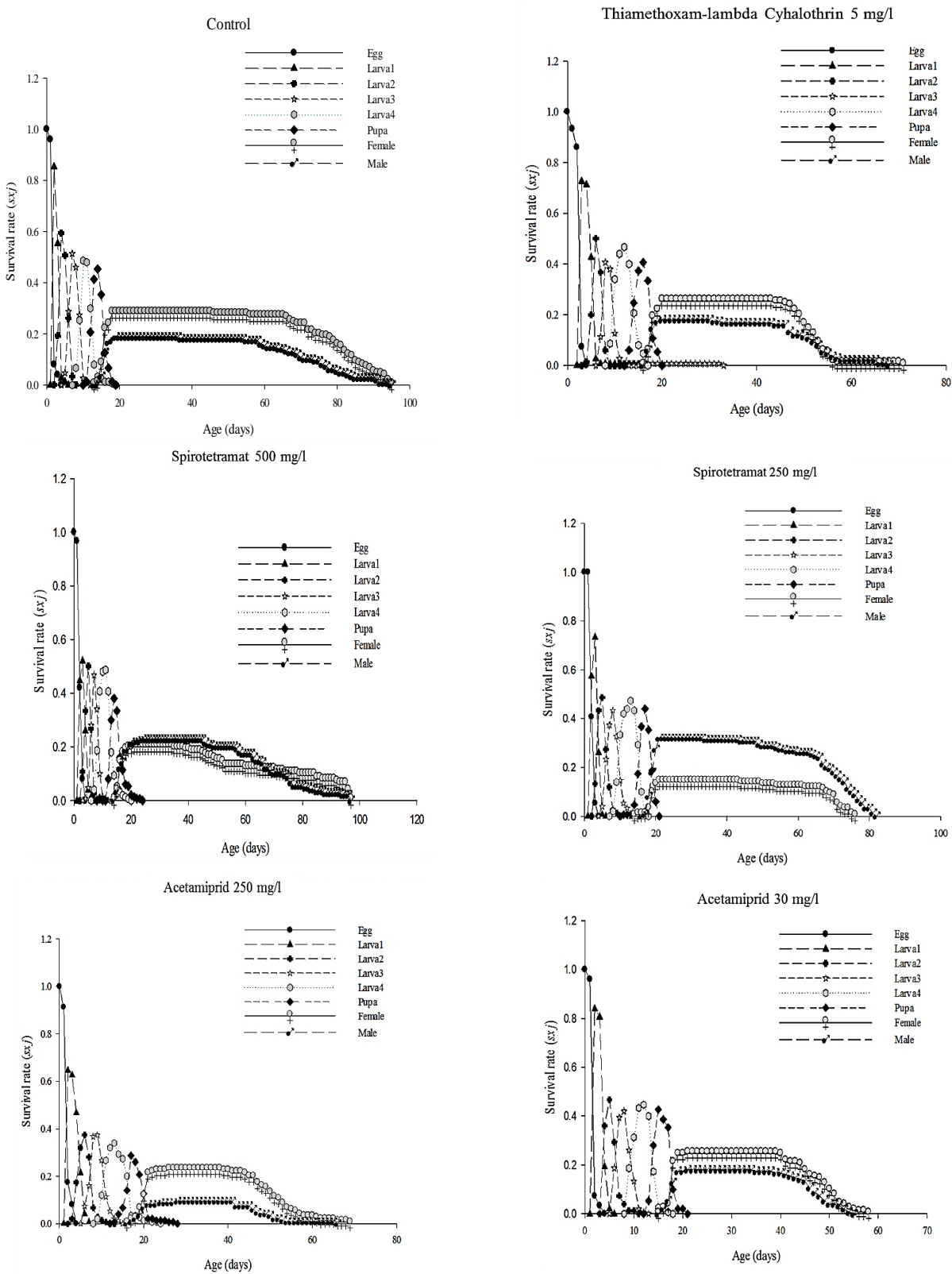


Figure 1. Age-stage specific survival rate (S_{xj}) of *O. conglobata contaminata*

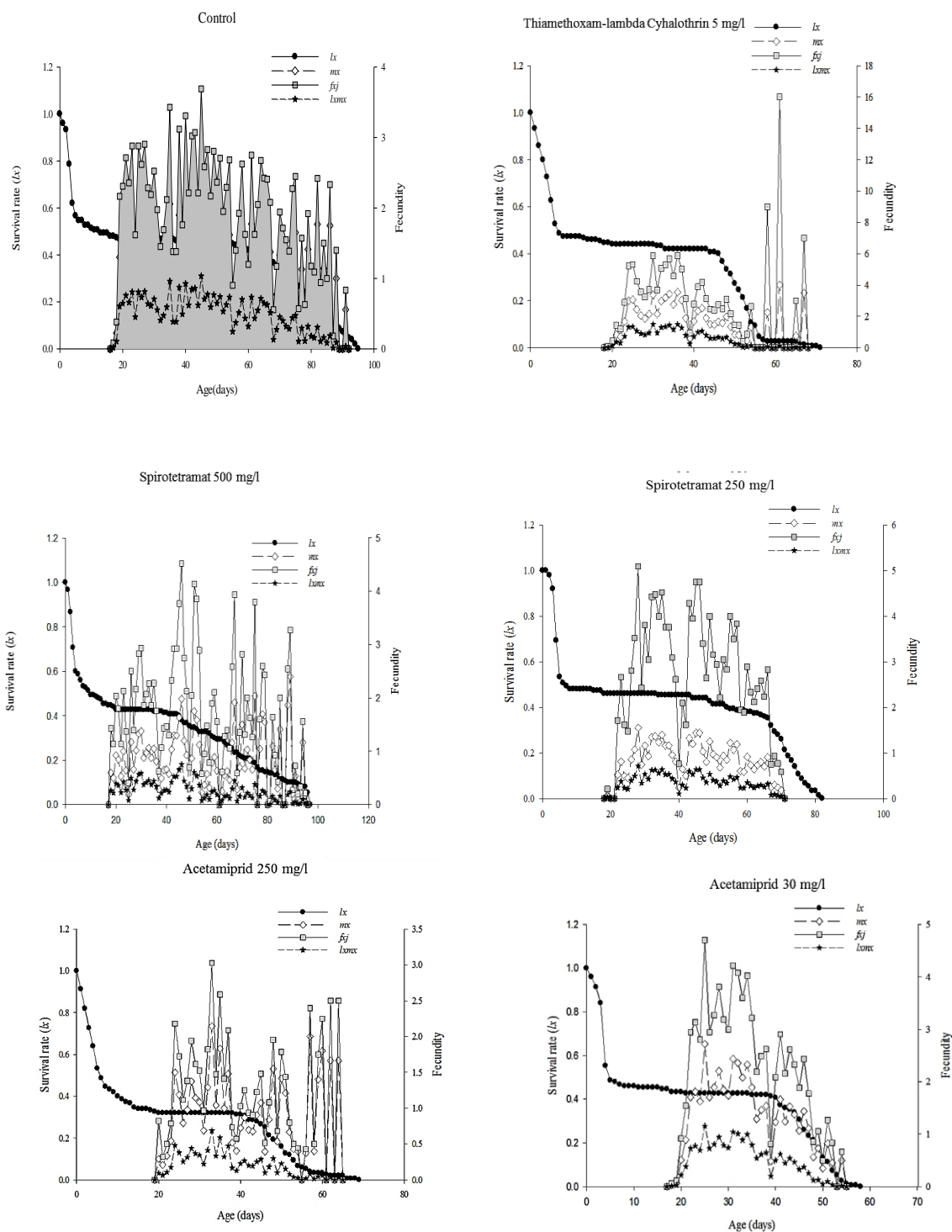


Figure 2. Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *O. conglobata contaminata*

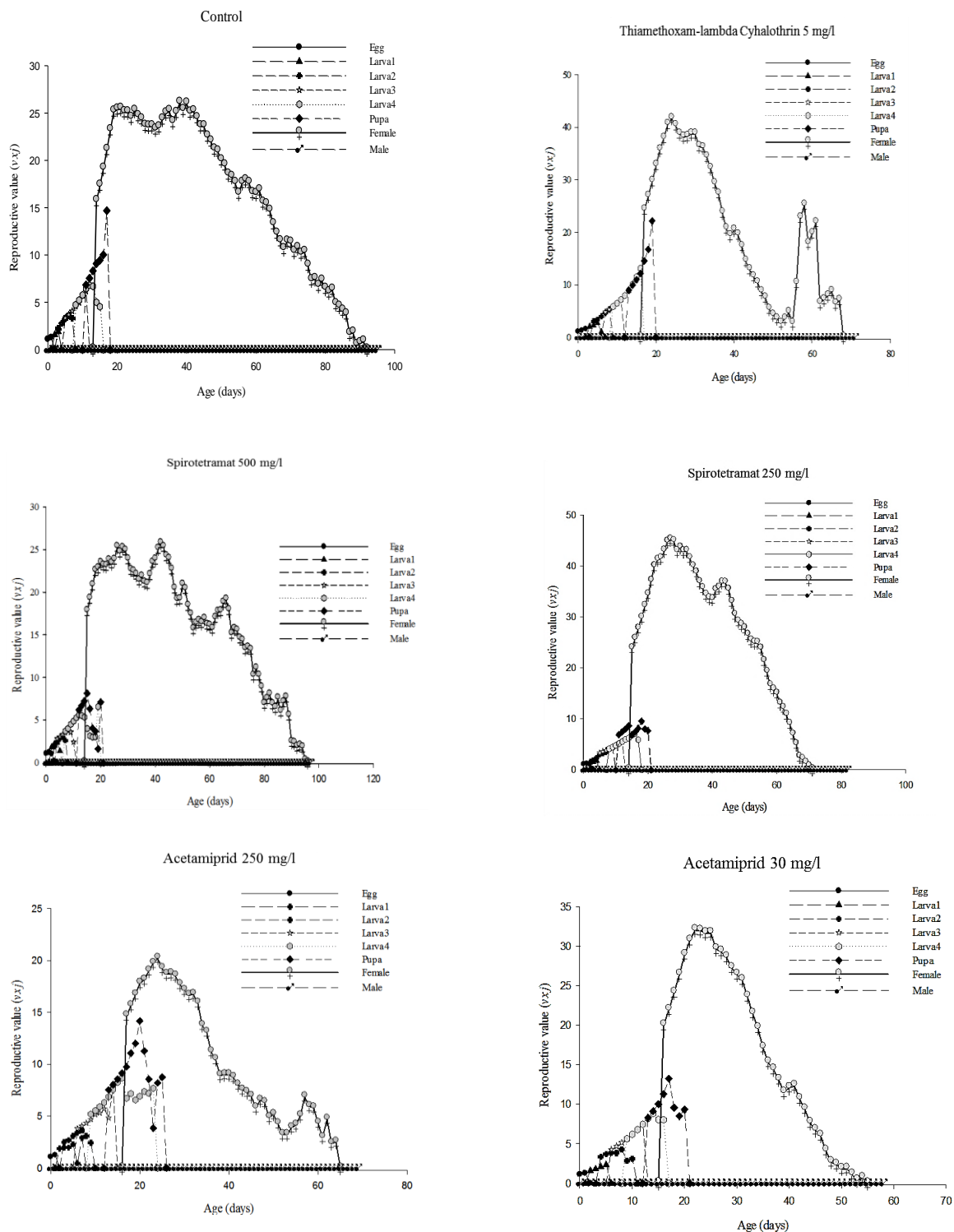


Figure 3. Age-stage specific reproductive value (v_{xj}) of *O. conglobata contaminata*

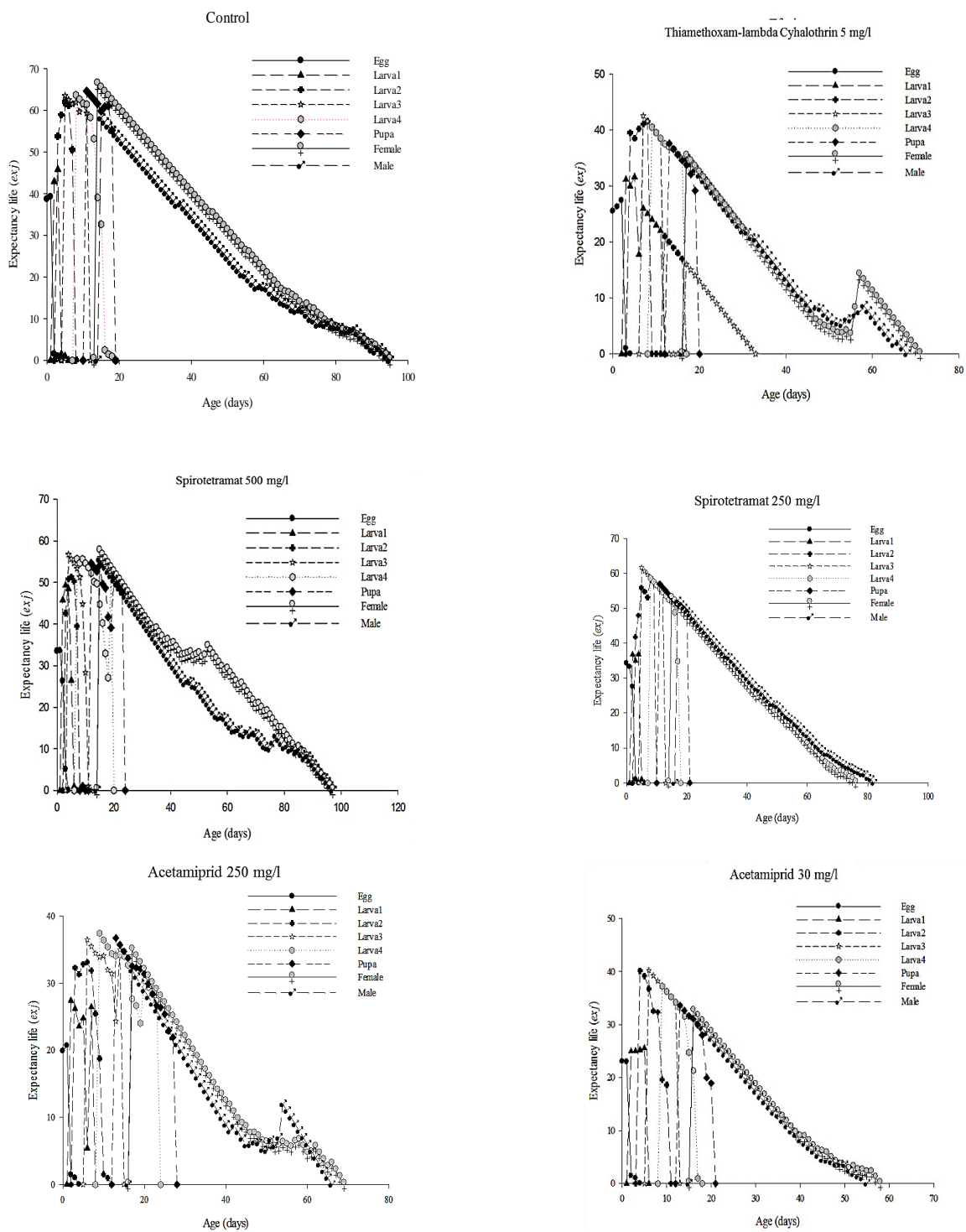


Figure 4. Age-stage specific life expectancy (e_{xi}) of *O. conglobata contaminata*

5. Discussion

Common pistachio psylla is one of the key pests of pistachio. Chemical control has been conducted by the farmers and gardeners to reduce the damages arising from this pest. There are several reports on the effects of different insecticides on the efficiency, physiology and behaviour of natural enemies [21, 22]. The effects of sub-lethal doses of insecticides are likely to be reducing longevity [12, 23], growth rate [24], fertility [25], reproduction [26, 27, 28], sex ratio change [24], change in behaviour, such as feeding [29], searching behaviour [29, 30], and oviposition [31] sterilization and repellency [21]. The studies conducted so far on the biological effects of pesticides on natural enemies indicate the high significance of the issue. The study conducted by Mostafaloo et al [32] indicated that chlorpyrifos affects the survival and development of eggs and fourth instar larvae of *C. montrouzieri*. Moreover, in the study conducted by Alimohammadi Davarani et al (33), it has been indicated that hexaflumuron increased the incubation period of *Hippodamia variegata* in comparison to spirotetramat and control. The oviposition period in *Adalia bipunctata* also increased by using hexaflumuron [34]. Liu and Chen [25] have indicated that phenoxycarp affects the egg stage of *Chrysoperla rufilabris* (Neuroptera) and the total growth period from egg to adult in the treatments was 2.6 to 4.2 longer than which it treated with water. The studies have indicated that the longest developmental period of *Habrobracon hebetor* was related to by sublethal concentration of sirinol with a contact method [36] and the first instar larval period has increased in all treatments. The most effective treatment in increasing of the first instar duration

was observed in acetamiprid 250 mg/l. The study conducted by Alimohammadi et al [33] also indicates an increase in the first instar larval period in *Hippodamia variegata* by hexaflumuron in comparison to spirotetramat and control. Another study has indicated that the thiamethoxam increases the first instar larval period in *Chrysoperla carnea* [37]. In this study, the larval period has increased in all treatments except for spirotetramat 500 mg/l which is consistent with the results of research conducted by Zeinadini et al [15]; acetamiprid increased the larval period of *H. variegata* in comparison to the control [9.11]. In investigating the lethal and sub-lethal effects of spirotetramat on the eggs of *Menochilus sexmaculatus* Fabricius at concentrations of 25, 50 and 100 ppm, it has been indicated that the treatment of eggs, significantly increased the larval period [13]. The results of this study indicated that there is a significant reduction in the longevity of adults in the treatments compared to the control. The sub-lethal effects of ethion and imidacloprid on *C. montrouzieri* showed the reducing effect of these insecticides on the longevity of ladybirds [38]. The study conducted by Rezaei et al on *H. hebetor* indicated the increased longevity of females which treated with Tondexir in a contact method, and the highest longevity of male was reported for sublethal concentration of Neem Azal in a contact method [39]. The results have also indicated that the intrinsic rate of natural increase and the finite capacity for increase of the ladybird have reported to have largest decrease in acetamiprid 250 mg/l in comparison to the control [38]. Keshtkar et al [38] have stated that the intrinsic rate of natural increase in *C. montrouzieri* reduced in ethion and imidacloprid. The study conducted by Iran

Nejad et al [40] indicate the negative effect of common fumitory (*Fumaria officinalis*), thymes (*Thymus vulgaris*) extract and hexaflumuron on the intrinsic rate of natural increase of *C. carnea*. Rezaei et al have stated that the r_m of *H. hebetor* has reduced in thiacloprid, azadirachtin and the red pepper extract, Abamectin and Proteus in contact and digestive methods [41, 42]. The effects of the sublethal dose of thiamethoxam-lambda cyhalothrin on the first instar larvae of green lacewing showed a decrease in values of r_m and λ in comparison to *Anethum graveolens* and *Rubia tinctorum* extracts. The intrinsic rate of natural increase decreased in *H. variegata* treated with acetamiprid and spirotetramat which the results are consistent with the present study [15].

In this study, the net reproduction rate (R_0) and the gross reproduction rate (GRR) in the treatments showed a significant difference with the control. The results of the study conducted by Ahmadi et al [43] showed a decrease in R_0 and GRR in *C. montrouzieri* in imidacloprid and abamectin. In this study, the mean generation time in the treatment of spirotetramat 250 mg/l shows the highest increase compared to the control which is consistent with the results of research conducted by Zeinadini et al. A research by Amin et al indicated the sublethal concentration of spirotetramat, fenitrothion and chlorpyrifos reduced the growth parameters of *O. conglobata contaminata* [14]. In the present study, the lowest age-specific fecundity of the ladybirds was observed in thiamethoxam-lambda cyhalothrin 5 mg/l. fecundity is the most sensitive biological indicator that is affected by insecticides [44]. The fecundity rate of *M. sexmaculatus* decreases sharply with increasing

concentration of pyriproxyfen [45]. Spirotetramat 500 mg/l prolongs the adult pre-oviposition period of the ladybird. The effect of pirimicarb, buprofezin and pymetrozine on the reproduction parameters of *Coccinella undecimpunctata* has been investigated which only pymetrozine increased the pre-oviposition period [46]. The lethal and sublethal concentrations of spirotetramat on the eggs of *M. sexmaculatus* have been investigated. It has significantly reduced the egg hatching percentage and emerging of the adults, and it has increased the larval and pre-oviposition periods [13]. In this study, the shortest oviposition period was observed in spirotetramat 500 mg/l. Keshtkar et al [38] have stated that ethion and imidacloprid reduced the oviposition period of *C. montrouzieri*.

6. Conclusion

The results showed that the adverse effects of spirotetramat were less than thiamethoxam-lambda cyhalothrin and acetamiprid on *O. conglobata contaminata*. One of the reasons can be attributed to the knock down effect of thiamethoxam-lambda cyhalothrin and acetamiprid on the target pest and also non-target insects such as natural enemies. These effects will be greater, especially in thiamtoxam-lambda insecticide, which has a pyrethroid compound in the active ingredient. It is suggested that in addition to paying attention to the application method of pesticides, the appropriate time of spraying, it is necessary to use the selective insecticides for conservation of natural enemies such as *O. conglobata contaminata*.

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