

The Impact of the Pesticides Abamectin and Spirotetramat on Biological Parameters of the Coccinellid Predator *Cheilomenes Sexmaculata* (Fabricius) Via Egg Exposure

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Information	Abstract
<p>Article Type: Original Article</p>	<p>Introduction: Due to the presence of pesticide residues in agricultural products, the security and safety of foods have taken on close attention. <i>Cheilomenes sexmaculata</i> (Fabricius) (Coleoptera: Coccinellidae) is a principal biological control agent in pistachio orchards, especially against <i>Agonoscena pistaciae</i> Burckhardt and Lauterer (Hemiptera: Psyllidae) that is the most damaging pest of pistachio. It is an effective predator to be used as a bio-control agent, which can reduce the use of pesticides in integrated pest management (IPM) program.</p> <p>Materials and Methods: In this project, the side effects of spirotetramat and abamectin were evaluated on <i>C. sexmaculata</i> by dipping the egg stage into the pesticide concentrations. Spirotetramat was tested at three concentrations of 100, 50, and 25 mg/L, representing 2/1, 1/1, and 1/2 of the maximum field recommended concentration (MFRC). Abamectin was also tested at a dilution series of 1/1, 1/2, 1/4, 1/8, and 1/16 of its MFRC, corresponding to 9, 4.5, 2.25, 1.12, and 0.56 mg/l, respectively.</p> <p>Results: Egg hatching was significantly reduced when eggs were exposed to all concentrations of insecticides except at 1/2 MFRC of spirotetramat. The results of the present project revealed that spirotetramat caused significant mortality only on first-instar larvae of <i>C. sexmaculata</i>. Pre-oviposition period, percentage of adult emergence, and developmental period of predator larvae were significantly affected by all tested concentrations of both insecticides. Egg hatching was significantly reduced when eggs were exposed to each tested insecticide at all concentrations of both insecticides except 1/2 spirotetramat.</p> <p>Conclusion: This research highlighted the importance of toxicity risk assessments, including lethal and sub-lethal effects, to obtain a more accurate estimation of the compatibility of insecticides in current IPM programs. Abamectin was not compatible with biocontrol agent <i>C. sexmaculata</i> and could not be used in IPM programs due to its strong lethal and sub-lethal effects. Spirotetramat was considered to be less harmful on <i>C. sexmaculata</i> than abamectin and could be compatible with augmentative releases of the coccinellid, <i>C. sexmaculata</i>.</p>
<p>Article History: Received: 23 Nov. 2018 Accepted: 27 Jan. 2019 DOI: 10.22123/ phj.2019.182680.1030</p>	
<p>Keywords: Biological Control <i>Cheilomenes Sexmaculata</i> Insecticide Psyllid Sub-Lethal Effects</p>	
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► Please cite this article as follows:

Azod F, Shahidi-Noghabi Sh, Mahdian K. The impact of the pesticides abamectin and spirotetramat on biological parameters of the coccinellid predator *Cheilomenes sexmaculata* (Fabricius) via egg exposure. *Pistachio and Health Journal*. 2019; 2 (1): 62-70

1. Introduction

The common pistachio psyllid, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae), is one of the most damaging pests of pistachio (*Pistacia vera*) [1]. Chemical control is the prevalent method in the management of this pest. The extensive use of insecticides for pest control may cause undesirable effects on non-target organisms, such as biological control agents. Therefore, evaluation of their possible effects on the survival and beneficial ability of natural enemies is important in order to find selective insecticides for incorporation into IPM¹ programs [2]. Insecticides may either cause the death of biological control agents (lethal effects) or modify some of their biological parameters without killing them (sub-lethal effects) [3]. Coccinellids usually play an important role in IPM programs in numerous agroecosystems [4]. Both larval and adult stages of all coccinellid predator species provide significant biological control services feeding on different soft-bodied pests, such as aphids, whiteflies, mites, as well as lepidopteran eggs and larvae [5].

C. sexmaculata Fabricius (Coleoptera: Coccinellidae) is an important biological control agent in various parts of the world, in East Asia in particular [6]. Its activities as a predator have been reported on different species of aphids and psyllids. One of the main purposes of IPM strategies is the combination of selective pesticides with biological control agents, i.e., predators and parasitoids [7]. Therefore, the evaluation of side effects of pesticides on natural enemies, both lethal and sub-lethal effects, is crucial prior to IPM programs application [8, 9]. The ambimobile insecticide spirotetramat is a

tetramic acid derivative used to control sucking insects. This pesticide inhibits the lipid biosynthesis, particularly in juvenile stages [10]. Abamectin is a naturally derived acaricide/insecticide isolated from the fermentation of the soil microorganism, *Streptomyces avermitilis* [11].

In the process of pesticide registration, assessment of their compatibility in IPM programs usually begins with a calculation of their acute toxicity, which offers critical information on the risk they possibly will cause on natural enemies [12]. However, the importance of sub-lethal effects of insecticides on different biological parameters of predators and parasitoids has been documented [13]. The few studies available have categorized this lipid biosynthesis inhibitor being harmless to other natural enemies, such as the predators *Episyrphus balteatus* (de Geer) (Diptera: Syrphidae), *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) [14], and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) [15]. Susceptibility to abamectin has been shown for several ladybeetle species, including *Harmonia axyridis* Pallas, *Cryptolaemus* sp., *Cycloneda sanguinea* Linnaeus, as well as *Stethorus punctum* (LeConte) larvae and adults [16- 18]. The side effects of these two insecticides have not been tested on the eggs of *C. sexmaculata* before. In our previous research, the side effects of spirotetramat and abamectin were assessed, when lady beetle was exposed to insecticides through the ingestion of contaminated prey. Previous results demonstrated no acute mortality to ladybeetles upon exposure to 2/1, 1/1, and 1/2

¹ Integrated Pest Management (IPM)

of the spirotetramat MFRC². Abamectin was very harmful with high acute toxicity at 1/1, 1/2, 1/4, and 1/8 of its MFRC; thus, this pesticide could not be recommended for use in an IPM program [19]. In this study, both the lethal and sub-lethal effects of spirotetramat and abamectin (two insecticides commonly used in many pistachio orchards) on *C. sexmaculata* are examined by treating the egg stage. The results may provide a compatibility measure of spirotetramat and abamectin in the current IPM programs with *C. sexmaculata*.

2. Material and Method

2.1. Insect

C. sexmaculata was reared in the ventilated plastic boxes (20 × 25 × 10 cm) at 25± 2°C, 65± 5% RH and a photoperiod of 16:8 (L:D). The beetles were provided with fresh pistachio leaves containing *A. pistaciae* as food. To prevent fungal growth, leaves were changed daily, and those containing beetle eggs were separated and transferred to Petri dishes. Due to the cannibalism behavior of the predatory larvae, after egg hatching, the larvae were transferred separately to Petri dishes. After two generations of breeding and feeding on *A. pistaciae* for adaptation, *C. sexmaculata* were used for experiments.

2.2. Insecticides

Commercial formulations of spirotetramat (Movento, 10% SC, Bayer Crop Science) and abamectin (Vertimec 1.8% EC, Agriphar, Belgium) were used. The former was investigated at a dilution series of 2/1, 1/1, and 1/2 of its MFRC corresponding to 100, 50, and

25 mg/L, respectively. Also, the latter was studied at a dilution series of 1/1, 1/2, 1/4, 1/8, and 1/16 of its MFRC that corresponds to 9, 4.5, 2.25, 1.12, and 0.56 mg/L, respectively.

2.3. Effect of Insecticides on Mortality and Biological Parameters of *C. Sexmaculata*

The bioassay was carried out using a completely randomized design with 20 treatments and 3 replicates. For this purpose, leaves containing one-day eggs of *C. sexmaculata* were dipped for 5 seconds into each insecticide with the concentrations as mentioned earlier. Later, treated eggs were exposed to the air for an hour to dry. The treated eggs were placed in Petri dishes of 90 mm diameter on a piece of filter paper and kept in the insectarium at 25 ± 2°C and 65 ± 5 % RH. Subsequently, eggs were checked daily, and after hatching, they were kept individually to prevent cannibalism. Larvae were provided with fresh pistachio leaves containing *A. pistaciae* as a food until they became adult. During this experiment, the mortality of different larval stages was recorded. Also, the percentage of egg hatching, the developmental time of immature stages (larvae, pre-pupae, and pupae), the rate of adult emergence, and the pre-oviposition period were measured. Distilled water was used as control. The experiment was repeated thrice independently (20).

2.4. Data Analysis

Data on mortality were corrected using Abbott's formula (21). Biological parameters, such as egg hatching, the developmental period of immature stages (larvae, pre-pupae, and pupae), the rate of adult emergence, and the pre-oviposition period were tested for normality.

² Maximum Field Recommended Concentration (MFRC)

Data were analyzed via SPSS software, and means with significant differences were separated by the analysis of variance (ANOVA) followed by a Tukey test at $P < 0.05$.

4. Results And Discussion

4.1. Egg Hatching of *C. Sexmaculata*

Egg hatching was significantly reduced when eggs were exposed to each tested insecticide ($F = 358.6$; $df = 8, 18$; $P < 0.05$). Spirotetramat had no effects on egg hatching at 1/2 of its MFRC; however, 8% and 16% reductions were observed when eggs were treated with 1/1 and 2/1 of their MFRC, respectively, as compared to control. In contrast, no egg was hatched in abamectin treated eggs at 1/1, 1/2, and 1/4 of

their MFRC while only 15% of eggs were hatched at 1/8 MFRC. This insecticide was very toxic to *C. sexmaculata* and caused sub-lethal effects even at a very low concentration (1/16 MFRC). Therefore, further experiments with abamectin were conducted at 1/16 MFRC, which caused a 39% reduction in egg hatching as compared to control (Table 1). As a result, it could be suggested that abamectin is not compatible with biocontrol agent *C. sexmaculata* and could not be used in IPM programs due to its strong lethal and sub-lethal effects. Currently, spirotetramat can be classified as practically non-toxic to birds and mammals on an acute basis and also non-toxic to honeybees based on acute oral and contact studies [21].

Table 1. Effect of different concentrations of the insecticides on egg hatching (mean percentage \pm SE) of *C. sexmaculata* when eggs were treated via dipping method

Concentration	Spirotetramat MFRC ¹			Abamectin MFRC ²					Control
	1/2	1/1	2/1	1/16	1/8	1/4	1/2	1/1	-
Egg hatching (%)	76.66 \pm 1.30a	68.33 \pm 1.66b	60 \pm 1.66c	61 \pm 3.78c	15 \pm 3.30d	0 \pm 0e	0 \pm 0e	0 \pm 0e	76.66 \pm 1.66a

Data are expressed as mean egg hatching \pm SE based on three biological replicates. Percentages followed by different letters in each column are significantly different ($P < 0.05$, Tukey test).

1 MFRC for spirotetramat= 50 mg/L

2 MFRC for abamectin= 9 mg/L

Abamectin is known to act on the nervous system of arthropods and has been indicated as an allosteric modulator of a glutamate-gated chloride channel (GluCl) [22]. Spirotetramat is a ketoenole, a derivative of tetroneic acid; it acts mostly by ingestion, where it inhibits lipogenesis that leads to adiminution of growth regulators and fertility [23]. Although both insecticides are vital to the survival of the insect, abamectin with high K_{ow} (4.00) leads to high mortality of eggs,

even in concentrations lower than MFRC [24]. Similar results have been observed in other studies; for instance, abamectin and spirotetramat+ imidacloprid reduced egg hatching of *Orius insidiosus* (Hemiptera: Anthocoridae) [20]. Also, it has been reported that spirotetramat caused 37.6% egg mortality on *Neoseiulus fallacises* (Acari: Phytoseiidae) [23]. Likewise, egg mortality by spirotetramat has been reported on *Galendromus occidentalis*

[25]. Indeed, our results demonstrated significant differences in the viability of eggs among the treatments and control. However, in contrast to our results, *Chrysoperla externa* (Hagen, 1861) egg viability was not affected by abamectin [26]. It is worthwhile to note that the reduction in viability of *C. sexmaculata* eggs caused by abamectin and spirotetramat may result from their high partition coefficient (K_{ow}) values (4.00 and 2.51, respectively). High K_{ow} values confer higher lipophilicity and facilitate penetration of a greater amount of the product through the chorion and its translocation to its site of action [27]. In our previous research, no significant effect on egg hatching has been reported when insecticide-treated prey was ingested by lady beetle [19]. Moreover, it has been stated that more than 90% of the *C. sexmaculata* eggs were hatched at 1/16 MFRC when ladybeetle was exposed to the insecticide by ingestion of abamectin-treated prey, while fewer eggs (61%) were hatched once they were directly exposed to abamectin at an identical concentration. These results point out the fact that the contact toxicity of abamectin on *C. sexmaculata* eggs compare to the ingestion of contaminated prey had a more severe effect on egg hatching [19].

4.2. Mortality of Different Larval Instars of *C. Sexmaculata*

Results obtained from this research, clearly indicated that abamectin had more harmful effects against *C. sexmaculata* compared to spirotetramat; since no egg hatching was observed in higher concentrations of abamectin, the tests were conducted at 1/16 MFRC. Spirotetramat at all tested concentrations and

abamectin at 1/16 MFRC caused significantly higher mortality only on the first-instar larvae of *C. sexmaculata* as compared to control ($F= 4.03$; $df= 4, 10$; $P< 0.05$). Nonetheless, no mortality was observed in the second, third, and fourth-instar larvae of *C. sexmaculata* at the concentrations tested with each insecticide (Table 2). Spirotetramat is a keto-enol, a derivative of tetrionic acid, which mostly targets hemipterans. The active ingredients of spirotetramat act on the biosynthesis of lipids causing inhibition of lipid biosynthesis. According to the U.S. Environmental Protection Agency [22], the basic risk to bees seems to be low-based on acute oral and contact experiments with honey bees. The results of the present project revealed that spirotetramat at 2/1, 1/1, and 1/2 MFRC caused significant mortality only on first-instar larvae of *C. sexmaculata*. Similarly, 100% mortality was observed in the first instar nymphs of *O. insidiosus* emerged from eggs and treated with abamectin within hours of evaluation [20]. Results from our previous research, lady beetle exposed to the spirotetramat-treated prey (*A. pistaciae*), compared to the present results indicated that ingestion had a more adverse effect on survival of lady beetles than contact of the eggs. Moreover, no acute toxicity was observed as spirotetramat was used via egg exposure, and at 1/1 MFRC, only 8.9% mortality was detected in the first-instar larvae of *C. sexmaculata*. In great contrast, abamectin had acute toxicity in both egg contact method (present research) and the ingestion of treated prey, as described in the former report [19].

Table 2. Effect of different concentrations of the insecticides on mortality (mean percentage± SE) of *C. sexmaculata* larvae when eggs were treated via dipping method

Treatment	Mortality (%)			
	L1	L2	L3	L4
Spirotetramat				
1/2 MFRC1	13.03± 0.26B	0	0	0
1/1 MFRC	14.28± 0.00B	0	0	0
2/1 MFRC	16.23± 0.42B	0	0	0
Abamectin				
1/16 MFRC2	8.58± 2.8AB	0	0	0
Control (Water)	4.4± 1.2A	0	0	0

Data in each column followed by different letters are significantly different ($P < 0.05$, Tukey test)

1 MFRC for spirotetramat = 50 mg/L

2 MFRC for abamectin = 9 mg/L

4.3. Biological Parameters of *C. Sexmaculata*

As shown in table 3, pre-oviposition period was significantly increased at all concentrations of both insecticides ($F = 52.9$; $df = 4, 10$; $P < 0.05$) except at 1/2 MFRC of spirotetramat as compared to control. Also, the percentage of adult emergence was significantly affected compared to control. However, the greatest impact was found in 1/1 and 2/1 MFRC of spirotetramat with 23 and 25% reduction compared to control, respectively ($F = 148.02$; $df = 4, 10$; $P < 0.05$). The developmental period of pre-pupae ($F = 2.14$; $df = 4, 10$; $P > 0.05$) and pupal period ($F = 1.32$; $df = 4, 10$; $P > 0.05$) were

not affected by insecticides as compared to control. However, the difference was verified in the developmental period of the larval stage, which was affected by both insecticides, and the longest larval development time was observed at 2/1 MFRC of spirotetramat with an increase of 2.98 days compared to control ($F = 29.38$; $df = 4, 10$; $P < 0.05$). Similar to the present results, abamectin and spirotetramat+ imidacloprid affected the duration of the nymphal period and reduced nymphal survival of *O. insidiosus* (20) as spirotetramat is a ketoenole, a derivative of tetroneic acid and acts by inhibiting lipogenesis that leads to a diminution of growth regulators and fertility.

Table 3. Effect of insecticides on biological parameters of *C. sexmaculata* when eggs were treated via dipping method

Treatment	Concentration (mg/L)	Developmental Period of Larvae (day)	Developmental Period of Pre-Pupae (day)	Developmental Period of Pupae (day)	Adult Eclosion (%)	Pre-Ooviposition Period (day)
Spirotetramat	1/2 MFRC1	8.33± 0.33bc	1.04± 0.025a	2.85± 0.04a	76.7±1.7b	4.0± 0.0a
	1/1 MFRC	8.6± 0.30bc	1.06± 0.03a	2.86± 0.03a	70.7± 0.7a	5.3± 0.3b
	2/1 MFRC	9.0± 0.0c	1.10± 0.00a	2.86± 0.04a	68.4± 0.8a	7.6± 0.3d
Abamectin	1/16 MFRC2	7.95± 0.016b	1.06± 0.03a	2.81± 0.003a	89.2±0.4c	6.6± 0.2c
Control	Distilled water	6.02± 0.02a	1.04± 0.02a	2.85± 0.04a	93.5±0.1c	4.0± 0.0a

Data are expressed as mean biological parameters± SE based on three biological replicates. Percentages in each column followed by different letters are significantly different (P<0.05, Tukey test).

1 MFRC for spirotetramat= 50 mg/L

2 MFRC for abamectin= 9 mg/L

5. Conclusions

These results may provide valuable information to find a more precise time and method for field application of the tested insecticides given the IPM program in order to reduce pesticide use to provide safety for humans and the environment. The side effects caused by abamectin and spirotetramat could be a consequence of the combination of delayed toxicity following egg absorption. In conclusion, according to the results, spirotetramat by dipping the eggs is considered to be less harmful on *C. sexmaculata* than abamectin and can be compatible with augmentative releases of the coccinellid, *C. sexmaculata*. However, it is believed that spirotetramat is safe when used at its MFRC, considering that it is also

metabolized and diluted before getting the natural enemies. It is also important to bear in mind that the coccinellids are only extremely exposed to the MFRC at the period of spraying. However, further testing under more field-realistic conditions may be useful as these would also take environmental persistence into account.

Conflicts of interest

We confirm that there are no conflicts of interest related to this study, and there has been no substantial financial support for this work that could have influenced its outcome.

Acknowledgments

The authors are grateful to the Vali-e-Asr University of Rafsanjan for financial support.

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