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Susceptibility of immature stages of a biocontrol agent, *Cheilomenes sexmaculata*, to imidacloprid and pyriproxyfen

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ABSTRACT- Chemical control is one of the important strategies in agricultural pest management because of easy operation, availability and fast control of pests. On the other hand, the environment has been exposed to various types of pesticides and pollutants which are growing daily. The persistent effects of residual pesticides commonly used cause numerous problems for non-target organisms especially parasitoids and predators. In this research, the effects of pyriproxyfen and imidacloprid on *Cheilomenes sexmaculata* (F.) (Coleoptera: Coccinellidae) were studied. The eggs of the ladybird beetle were treated with each pesticide in three concentrations by dipping method. Maximum field recommended concentration (MFRC=50 mg/L), 2/1 MFRC (to simulate multiple treatments) and 1/2 MFRC (to test the sublethal effects) were used for pyriproxyfen and 1/1 MFRC (140 mg/L), 1/2 MFRC (70 mg/L) and 1/4 MFRC (35 mg/L) were used for imidacloprid. The highest mortality of the first instar larvae was observed in imidacloprid at MFRC and pyriproxyfen at 2/1 MFRC. Also, maximum influence on fertility was observed at 2/1 MFRC of pyriproxyfen and MFRC of imidacloprid with 55% and 44% reduction compared to the control, respectively. Moreover, pyriproxyfen caused significant retardation on larval development at concentrations higher than 1/2 MFRC. Both insecticides initiated a significant effects on adult eclosion compared to the control and the greatest influence was observed by imidacloprid at 1/1 MFRC with 89% reduction in adult eclosion. Although results revealed that imidacloprid has higher acute toxicity to *C. sexmaculata*, further results obtained from the analysis of on the life cycle parameters of *C. sexmaculata* also indicated that pyriproxyfen also had toxic effects on this predator.

INTRODUCTION

Although biological agents are good candidates for the control of pest insects in integrated pest management (IPM) program, parasitoids and predators are often sensitive to pesticides and care should be taken using compatible pesticides. Consequently, loss of parasitoids and predators with lethal dose of pesticides causes the release of primary pests and also the emergence of secondary pests (Stanley and Preetha, 2016).

Ladybird beetles are beneficial insects in agricultural ecosystems and play an important role in controlling various pest insects such as psyllids, aphids, whiteflies, mealybugs, leafhoppers and ticks (Jacas and Urbaneja, 2010; Planes et al., 2013). The ladybird beetle *Cheilomenes sexmaculata* is one of the most efficient predators in biological control. High fertility and voracity of this ladybird beetle has made it an effective agent in biological control in the pest management (Mehrnejad et al., 2011). This ladybird beetle is a persuasive predator of aphids and other soft-bodied insect pests such as psyllids (Agarwala and Yasuda, 2000; Omkar and Pervez, 2002). Also, it is one of the potential predators of the common pistachio psylla, *Agonoscena pistaciae* Burckhardt and Lauterer (Hemiptera: Psyllidae), which is one of the most important pests of pistachio orchards causing adverse effects on the quantity and quality of pistachio products. The trees are sprayed regularly with various pesticides in order to control this pest's damages. The main focus of the IPM is incorporated pest control methods, but in most situations, biological control alone has not been effective enough and farmers have to use pesticides, which are harmful for non-target species. Biological control and chemical control are two important strategies in control of pest insects and their compatibility is essential for the success of IPM programs. In the last decade, studies have been done on biological and behavioral influences of insecticides on natural enemies (Schneider et al., 2008; Delpuech et al., 2012; Wrinn et al., 2012; Zotti et al., 2013). Currently, many researches have shown that in addition to the direct effects of pesticides, mortality rate, sublethal concentrations of pesticides can also influence the physiology and behavior of natural enemies (Johnson and Tabashnik, 1999). Sublethal concentrations of insecticides are able to reduce the life cycle parameters of insects such as growth (Vinson, 1974), fertility (Rodrigues et al., 2018;

Grosch and Hoffman, 1973), fecundity (Rezaei et al., 2007), change in sex ratio (Vinson, 1974) and consumption ratio (Desneux et al., 2006).

Imidacloprid is a fast-performing neonicotinoid pesticide in the chemical family of chloronicotinyl nitroguanidine (Kramarz and Stark, 2003), and it influences the insect nervous system by attaching to the acetylcholine binding sites, known as nicotinic acetylcholine receptors (nAChRs). Recently, this insecticide has commonly been used in many orchards and limited research has been done to evaluate its effects on *C. sexmaculata* (Khan et al., 2014).

Pyriproxyfen is an analogue of the juvenile hormone that is commonly used as insect growth regulator (IGR) against homopteran insect pests (Ware and Whitacre, 2004; Ishaaya and Horowitz, 1992). Only few studies have been conducted to evaluate the safety of pyriproxyfen on *C. sexmaculata*.

Considering the importance of *C. sexmaculata* as a predator and understanding improved tactics for control of pests, this research emphasized on the importance of toxicity risk estimation including lethal and sublethal effects to achieve a more accurate guesstimate of the compatibility of insecticides in current IPM programs. Both pesticides tested are frequently used, and their effects should be investigated on non-target organisms and biological agents. In this research, sublethal effects of imidacloprid and pyriproxyfen were investigated on egg hatching, development of immature stages, mortality of the first instar larvae, adult emergence and pre-oviposition period of *C. sexmaculata*, when eggs were exposed to the different concentrations of each insecticide. The aim of the current study was to assess the sublethal effects of a biorational insecticide (pyriproxyfen) and a conventional insecticide (imidacloprid) on immature stages of the zigzag lady beetle, *C. sexmaculata*, to perceive their effects on the mortality and reproduction of ladybird under controlled laboratory conditions.

MATERIALS AND METHODS

Insect Culture

Adults of ladybird *C. sexmaculata* were collected from pistachio trees (*Pistacia vera*) in a pistachio garden at Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran and were placed in plastic boxes (25×20×10 cm). The common pistachio psyllid, *Agonoscena pistaciae*, were obtained daily from a pistachio garden with no insecticide treatment and were transferred to the laboratory for the experiments. Predator was reared on the pistachio psylla in growth chamber at 25 ± 5 °C, 60% RH and (16h light/ 8h dark) photoperiodic conditions. Eggs (< 12 h) were chosen and monitored until they were adults.

Insecticides

Commercial insecticides consisting of imidacloprid (35% suspension concentration, kavosh Kimia) and pyriproxyfen (Admiral, 10% suspension concentration, Arista Life Science) were used to evaluate the effects on ladybird.

Bioassay for Insecticidal Activity

After a pre-test with each insecticide based on maximum field recommended concentration (MFRC) and selecting final concentrations for bioassay of pyriproxyfen and imidacloprid, different concentrations of the two insecticides were used (Medina et al., 2005). Pyriproxyfen was tested with 2/1 MFRC to simulate multiple treatments (100 mg/L) and 1/1 MFRC (50 mg/L) and 1/2 MFRC (25 mg/L) to test sublethal effects. Because high mortality was observed at 2/1 MFRC of imidacloprid, this concentration was not used for subsequent experiments and 1/1 MFRC (140 mg/L), 1/2 MFRC (70 mg/L) and 1/4 MFRC (35 mg/L) were tested. For each treatment, 60 eggs of predator (< 12 h) were selected randomly and dipped into certain concentrations of each pesticide for five seconds. The eggs were exposed to air for one hour until dried. Later, eggs were placed in Petri dishes (5 cm diameter) on a piece of filter paper and were kept in a growth chamber at 25 ± 5 °C, 60% RH and 16h light/ 8h dark. Each treatment was repeated in three separate replications and in each replicate 20 eggs were used. These 20 eggs were placed in four separate Petri dishes containing five eggs which were considered as the sample. All experiments were repeated three times and each time (repetition), there were four samples and for each sample, five eggs were used. Once the eggs were hatched, larvae were provided with fresh pistachio leaves containing *A. pistaciae* as food. The development of *C. sexmaculata* larvae were monitored daily from egg until they became adult. Fertility, mortality of the first instar larvae, adult emergence and pre-oviposition period (the period from adult emergence until egg laying) of *C. sexmaculata* were recorded for each sample. Then, mean values of these four samples of each replicate were used as final data to be analyzed. Distilled water was used as the control (Moscardini et al., 2013).

Data Analysis

The experiments were organized in a randomized complete design containing 7 treatments (control, three concentrations of pyriproxyfen and three concentrations of imidacloprid). Data were analyzed using SPSS 22 (2013) and average of parameters was compared using Duncan's multiple range test at the level of five percent.

RESULTS AND DISCUSSION

Effect of the Insecticides on Fertility of *C. Sexmaculata*

After 24 h, the number of eggs hatched was recorded as fertility. Results showed that fertility in both treatments was significantly different from the control ($F = 21.08$, $df = 6, 14$, $P < 0.05$). The highest effect on fertility was observed in pyriproxyfen at 2/1 MFRC (100 mg/L) with 55% reduction and imidacloprid at 1/1 MFRC (140 mg/L) with 44% reduction compared to the control.

Also, imidacloprid at 1/2 MFRC (70 mg/L) and 1/4 MFRC (35 mg/L), and pyriproxyfen at 1/1 MFRC (50 mg/L) and 1/2 MFRC (25 mg/L) showed 39%, 24%, 27% and 20% reduction in fertility compared to the control, respectively (Fig. 1).

Insecticidal Activity of the Insecticides on C. Sexmaculata

After 24 h, mortality of the first instar larvae in both treatments increased significantly compared to the control ($F = 45$, df = 4, 10, $P < 0.05$). The highest mortality in the first instar larvae was recorded for imidacloprid treated eggs, where 80%, 53% and 37% mortality was observed at 1/1, 1/2 and 1/4 MFRC, respectively whereas higher mortality with pyriproxyfen treated eggs at 2/1, 1/1 and 1/2 of MFRC were recorded as 64%, 22% and 10% mortality, respectively. No significant difference was observed in mortality of the first instar larvae at 1/2 and 1/1 MFRC of pyriproxyfen as compared with the control (Fig. 2). There was no significant mortality in other larval stages tested with each insecticide.

Effect of the Insecticides on Larval Duration of *C. sexmaculata*

As the effect of two insecticides on larval duration was compared, imidacloprid had no effect on larval duration of surviving *C. sexmaculata* compared to the control, which took 7 days. But, pyriproxyfen at 2/1, 1/1 and 1/2 MFRC caused significant retardation on larval development and larval duration was about 8 days, which was significantly higher than the control ($F = 1$, df = 4, 10, $P < 0.05$) (Fig. 3).

Effect of Insecticides on Adult Emergence of *C. Sexmaculata*

Both insecticides caused a significant effect on adult eclosion compared to the control ($F = 72$, df = 4, 10, $P < 0.05$). The greatest influence was observed with imidacloprid at 1/1 MFRC (140 mg/L), in which only 9% eclosion was observed. The higher effect was followed by 2/1 MFRC (100 mg/L) of pyriproxyfen (27% eclosion), 1/2 MFRC of imidacloprid (40%) and 1/4 MFRC of imidacloprid (62%). The lowest effect on adult eclosion was observed on *C. sexmaculata* at 1/1 and 1/2 MFRC of pyriproxyfen with 67% and 80% adult eclosion, respectively. In the control, 98% of adults emerged from the pupae (Fig. 4).

Effect of the Insecticides on Pre-oviposition Period of *C. Sexmaculata*

No significant effect was observed in pre-oviposition period of *C. sexmaculata* by exposure to either imidacloprid or pyriproxyfen as compared to the control which was 4 days for all treatments. But, no egg was laid by *C. sexmaculata* adult when exposed to imidacloprid at 1/1 MFRC (140 mg/L) and pyriproxyfen at 2/1 MFRC (100 mg/L) (Fig. 5).

Imidacloprid is an insecticide of neonicotinoid group and compared to pyriproxyfen, has a broader spectrum; therefore, it was expected that the negative effects of this insecticide on survival of immature stages of *C. sexmaculata* would be more severe than pyriproxyfen. Results showed that imidacloprid not only in the recommended concentration, but also at lower amounts affected the survival and development of *C. sexmaculata* eggs.

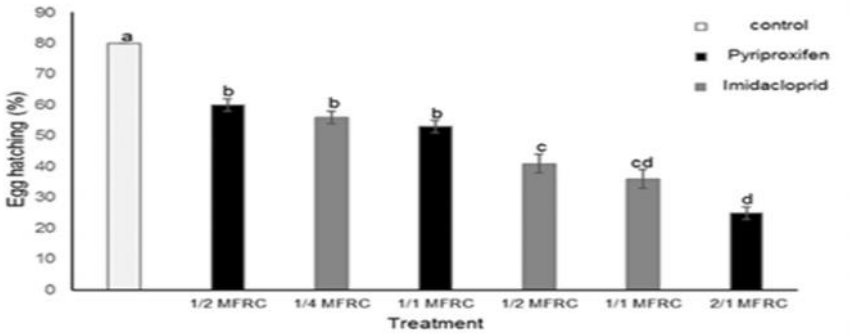

Fig. 1. The impact of the insecticides on egg hatching of *Cheilomenes sexmaculata* after 24 hours.
Means with the same letters are not significantly different (Dunkan's test, $P < 0.05$).
MFRC for pyriproxyfen = 50 mg/L
MFRC for imidacloprid = 140 mg/L

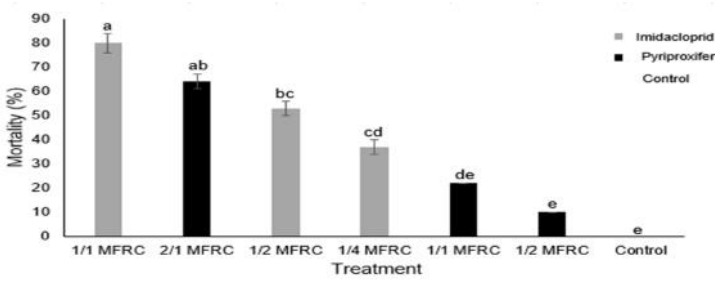

Fig.2. Mortality of the first instar larvae of *Cheilomenes sexmaculata* after eggs were exposed to the insecticides.
Means with the same letters are not significantly different (Dunkan's test, $P < 0.05$).
MFRC for pyriproxyfen = 50 mg/L
MFRC for imidacloprid = 140 mg/L

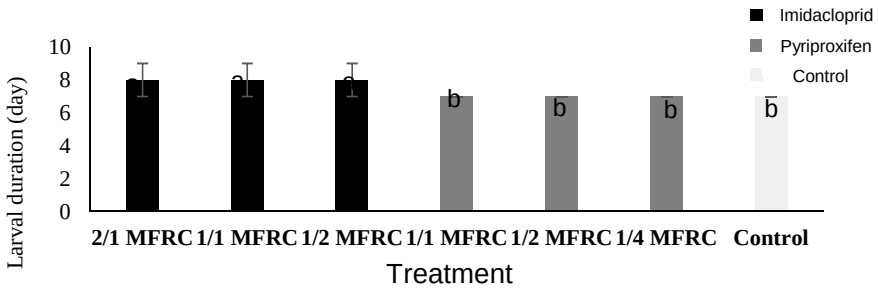

Fig. 3. Effect of the insecticides on larval duration of *Cheilomenes sexmaculata* when eggs were treated with each insecticide.
Means with the same letters are not significantly different (Dunkan's test, $P < 0.05$).
MFRC for pyriproxyfen = 50 mg/L
MFRC for imidacloprid = 140 mg/L

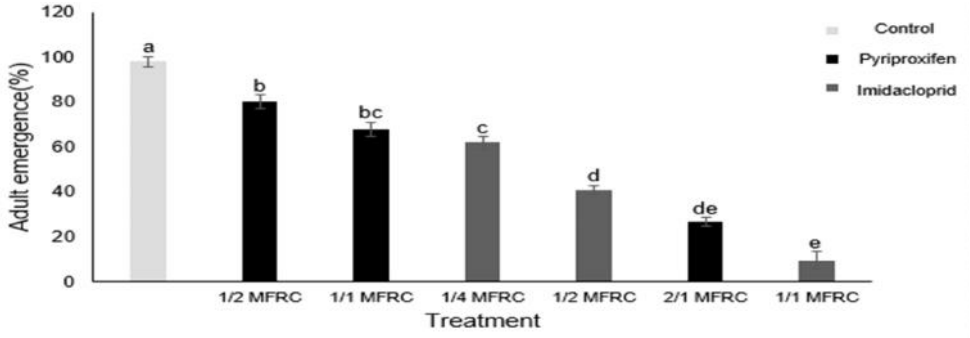

Fig. 4. Effect of insecticides on adult emergence of *Cheilomenes sexmaculata.*
Means with the same letters are not significantly different (Dunkan's test, $P < 0.05$).
MFRC for pyriproxyfen = 50 mg/L
MFRC for imidacloprid = 140 mg/L

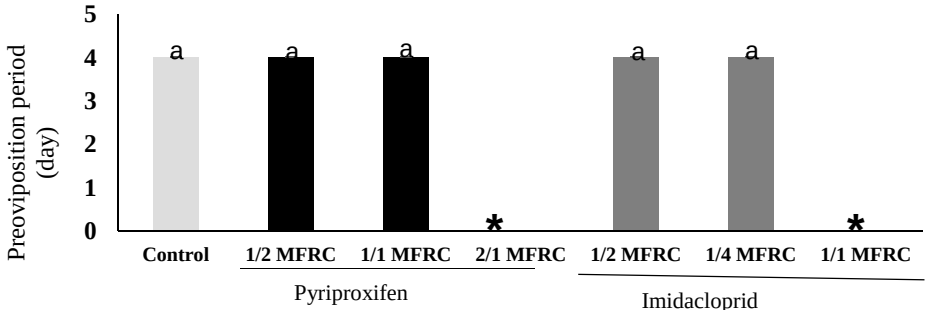

Fig 5. Effect of insecticides on pre-oviposition period of *Cheilomenes sexmaculata.*
Means with the same letters are not significantly different (Dunkan's test, $P < 0.05$).
MFRC for pyriproxyfen = 50 mg/L
MFRC for imidacloprid = 140 mg/L
*At these concentrations, no eggs were observed.

Pyriproxyfen is a juvenile hormone analog in insects and derivatives of fenoxicarb that is toxic to pests. It has been claimed that in field conditions, it has low risk to beneficial insects, but results of the current study showed dipping of the predator eggs in all tested concentrations of pyriproxyfen had great impacts on percentage of egg hatching, survival of first instar larvae, larval developmental time and also adult emergence of *C. sexmaculata*.

Based on previous observations, imidacloprid indicated acute toxicity against *C. sexmaculata* compared to pyriproxyfen. Also, chronic toxicity (sublethal effects) on immature stages of the predator *C. sexmaculata* was observed when eggs were exposed to pyriproxyfen or imidacloprid.

Although imidacloprid kills insects via ingestion or contact by disrupting the nervous system of an insect pest at larval and adult stages, based on previous research on capability of insecticides for penetration into the eggs of insects (Sohrabi *et al*., 2011), it is possible that imidacloprid was able to penetrate to the predator eggs and cause high toxicity.

In general, our results revealed that imidacloprid and pyriproxyfen in addition to lethal effects on eggs, had sublethal effects such as reduction in survival of the first instar larvae and percentage of adult emergence. Moreover, pyriproxyfen caused retardation in larval development even at lower concentrations than field recommended concentration. Likewise, ovicidal activity of pyriproxyfen has been documented when applied to eggs of codling moth (Charmillot et al., 2001) and diamondback moth (Oouchi, 2005).

Octan-water partition coefficients, log K_{ow}, seem to be a good indicator of ovicidal property among the chemical compounds. The log k_{ow} of pyriproxyfen (5.37) is higher than log k_{ow} of imidacloprid (0.57) and it was expected that pyriproxyfen has considerably more ovicidal activity compared to imidacloprid. The highest influence on fertility (55% reduction) was observed with pyriproxyfen (at 100 mg/L) whereas imidacloprid at higher concentration (140 mg/L) causes lower effect on fertility (44%). Although it is assumed that partitioning coefficients are not absolute interpreter of the toxicity of chemical compounds (Hoffmann et al., 2008), these results were consistent with the statement that there is a direct relationship between log K_{ow} and ovicidal activity.

In our experiment, at concentration of 1/1 MFRC (140 mg/L) of imidacloprid, only 36% of eggs were hatched. A comparable result regarding the reduction of egg hatching was observed on *Cryptolaemus montrozieri* when the eggs were dipped into imidacloprid at concentration of 500 ppm (lethal dose), in which only 46% of the eggs were hatched (Aghabaglou et al., 2013). In another study (Nazari et al., 2016), when the effect of imidacloprid was tested through the ingestion of insecticide-treated prey (*Agonoscena pistaciae*), 100% mortality was observed at 1/1 MFRC, while pyriproxyfen had no influence on the survival of *C. sexmaculata* even while fed on prey contaminated by 2/1 MFRC. Nevertheless, pyriproxyfen has indicated clear effects on prey ingestion, fertility and fecundity of *C. sexmaculata*. It is also worth mentioning that in previous research, imidacloprid even at very low concentration (1/100 MFRC = 1.4 mg/L) caused significant reduction on fecundity of *C. sexmaculata* when fed on contaminated prey (Nazari et al., 2016). Furthermore, effects of pyriproxyfen have been reported from the study of this insecticide on the survival and development of immature stages of *Chrysoperla rufilabris* (Chen and Liu, 2003). In contrast to what we imagine, pyriproxyfen has had more effects on predator at egg stage rather than later stages. These results are consistent with the results from Lio and Chent (2002) in which eggs of *C. rufilabris* were treated by pyriproxyfen, two higher concentrations of this pesticide (50 and 100 mg/L) reduced the eggs hatching by %33-%50. Besides, survival rate was also reduced. In another study, results on the influence of neonicotinoid on immature stages of the predator *Eriopis connexa* showed that this pesticide reduced egg hatching from 34 to %100 (Fogel et al., 2013). Likewise, the effect of two insect growth regulators (IGRs) and neonicotinoid insecticide on immature stages of parasitoid *Aphytis melinus* (Hem.: Aphelinidae) has been reported by Rill et al. (2008). They had shown that these IGRs did not affect survival or development of *A. melinus* adult. But in a recent study (Azod et al., 2016), spirotetramat, an inhibitor of lipid biosynthesis in insects, had no mortality even at 2/1 MFRC (100 mg/L) and showed compatibility with *C. sexmaculata*, when fed on the treated prey. Furthermore, these authors have reported the high effect of abamectin, even at concentration of 1/8 MFRC (1.12 mg/L) causing 100% mortality on *C. sexmaculata*.

Given these negative effects, especially imidacloprid, the compatibility of these insecticides with biological control programs of pistachio psylla is faced with hesitation. Results of these experiments demonstrated clearly that imidacloprid is not compatible with *C. sexmaculata* as a natural enemy of pests.

Present results along with previous findings lead to the fact that both methods of ingestion and contact toxicity bioassays with imidacloprid and pyriproxyfen influence the biological parameters of *C. sexmaculata* and in fact, the toxicity level depends on the growth stage of the insect exposed to insecticides. But to have more complete and comprehensive conclusions and evaluation of compatibility of these insecticides with *C. sexmaculata*, further research has to be conducted in a more close-to-natural environment such as greenhouse and field conditions.

CONCLUSIONS

According to the present results and considering the insect life cycle and developmental stages of insects and also use of pesticides in the right time, insecticides can definitely have better results in pest control management. More importantly, these results highlighted the need to consider both lethal and sub-lethal effects of pesticides because if the estimation was just established on survival, these products on plants in

the laboratory would be ranked as harmless. Nevertheless, further tests under more field-realistic conditions may be convenient as these take into account also the environmental persistence.

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حساسیت مراحل نابالغ یک عامل کنترل بیولوژیک، *Cheilomenes sexmaculata* به ایمیداکوپراید و پاپریپروکسی‌فن

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واژه‌های کلیدی:
مدیریت آفات کشاورزی*Cheilomenes sexmaculata*
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سمیت

چکیده- کنترل شیمیایی یکی از مهمترین استراتژی‌ها در مدیریت آفات کشاورزی می‌باشد، چرا که به راحتی در دسترس بوده و به‌خوبی آفات کشاورزی را کنترل می‌کند. از طرفی محیط زیست در معرض انواع مختلفی از آفتکش‌ها قرار می‌گیرد و میزان آلودگی آنها روی محیط زیست هر روز بیشتر می‌شود. همچنین، بقایای آفتکش‌های متداول که پیوسته مورد استفاده قرار می‌گیرد مشکلات زیادی روی موجودات غیرهدف بخصوص پارازیتوئیدها و شکارگرها بوجود می‌آورد. در این تحقیق، تاثیرات ایمیداکلوپراید و پایریپروکسی‌فن روی تخم کفشدوزک *Cheilomenes Sexmaculata* با روش غوطه‌وری تخم مطالعه شد. غلظت‌های پایریپروکسی‌فن شامل بالاترین غلظت توصیه‌شده در مزرعه (۵۰ mg/L)، دو برابر و یک دوم آن و برای ایمیداکلوپراید نیز بالاترین غلظت مزرعه(۱۴۰ mg/L)، یک دوم و یک چهارم آن استفاده شد. در بالاترین غلظت ایمیداکلوپراید تخمها تفریخ نشدند و بیشترین مرگ‌ومیر لارو سن اول نیز در این غلظت مشاهده شد. اما، هیچ مرگ و میری در سنین بالاتر لاروهای باقی مانده دیده نشد. دوبرابر غلظت توصیه‌شده پایریپروکسی‌فن و بالاترین غلظت ایمیداکلوپراید به‌ترتیب با ۵۵٪ و ۴۴٪ کاهش در باروری نسبت به شاهد بیشترین تاثیر را داشتند. همچنین، در یک دوم بالاترین غلظت توصیه شده پایریپروکسی‌فن و غلظت‌های بالاتر آن رشد لاروها تاخیر معنی‌داری داشت. بعلاوه، هر دو آفتکش در مقایسه با شاهد تاثیر معنی‌داری در کاهش خروج حشرات بالغ از پوسته شفیرگی داشتند، به‌طوری که در بالاترین غلظت ایمیداکلوپراید ۸۹٪ کاهش مشاهده شد. اگرچه نتایج به‌روشنی بیانگر این‌است که ایمیداکلوپراید اثرات سمی حاد بر *C. sexmaculata* داشته است، اما با ادامه آزمایشات روی پارامترهای جدول زندگی، نتایج نشان داد که پایریپروکسی‌فن نیز دارای اثرات سمی روی این شکارگر می‌باشد.