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Research Article

Laboratory evaluation of the toxicity of mineral oil, Diazinon, Malathion and Chlorpyrifos on ladybird, *Cryptolaemus montrouzieri* Mulsant (Col.: Coccinellidae)

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Abstract: Acute toxicity of the field recommended concentration of three conventional insecticides (Diazinon, Malathion and Chlorpyrifos) and mineral oil was evaluated on 3rd and 4th instar larvae and adults of *Cryptolaemus montrouzieri* Mulsant. The mortalities caused by the insecticides and mineral oil were significantly different. Diazinon and Malathion with 100% mortality showed the highest toxicity to the different stages of the ladybird. Chlorpyrifos and mineral oil caused less than 30% mortality, while mineral oil had the lowest harmful effect on this predator. Based on LC_{50} and LC_{90} values 24h after treatment, the male and female adults of *C. montrouzieri* were more susceptible to Diazinon (701 and 635; 1257 and 1194ppm) than to Chlorpyrifos (4238 and 4316, 5683 and 5480 ppm). Based on International Organization of Biological Control (IOBC) classification, Chlorpyrifos and mineral oil were classified as category 1 (harmless) and Diazinon and Malathion were placed in category 4 (harmful).

Keywords: Acute toxicity, bioassay, *Cryptolaemus montrouzieri*, organophosphate insecticide, mineral oil

Introduction

Predatory coccinellids are biological control agents of many pests including whiteflies (Aleyrodidae), aphids (Aphididae), mealybugs (Pseudococcidae), citrus cushion, scales and mites (Fernandez, 2015). *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is an efficient natural enemy of mealybugs and citrus cushion, *Pulvinaria aurantii* Cockerell and both larvae and adults of this predator feed on *P. aurantii* ovisacs and all developmental stages of mealybug (Aghabaglou *et al*., 2013; Halappa *et al*., 2013). This ladybird is used in most biological control programs of the mentioned pests around the world (Baskaran *et al*., 2002; Garcia and O'Neill, 2000; Kairo*et al*., 2013). Unfortunately, this predator is very sensitive to insecticides, like other natural enemies (Aghabaglou *et al*., 2013). The use of pesticides compatible with biological control agents and conservation of these agents are useful tools in integrated pest management (IPM) (Stark *et al*., 2004). On the other hand, the continuous use of insecticides has harmful effects on non-target arthropods because these useful species are more sensitive than their prey or host (Aghabaglou *et al*., 2013), which is due to basic

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ecological differences between them (Saber, 2001). The use of selective insecticides could reduce the adverse effects of these chemicals on the natural enemies (Galvan *et al*., 2005).

In recent decades, the use of organophosphate insecticides has disrupted activity of the natural enemies (Fernandez, 2015). These compounds are the most widely used insecticides in the world (Talebi Jahromi, 2011). Diazinon is a contact phosphate compound that is used both in the soil and on the plants. This insecticide is used against pests such as mites, aphids, etc. (EPA, 2000). Chlorpyrifos is a broad-spectrum insecticide that has contact, stomach and fumigant effects on the pest. It is applied in the soil as well as on the aerial parts of plants to control insects in alfalfa, cotton, rice, sugar beets, corn farms, citrus and fruit orchards (EPA, 2000). Malathion is one of the oldest and the most commonly used organophosphate insecticides that has a contact effect. It is used against pests of fruit trees, ornamental plants and Mediterranean fruit fly (Ware, 2000). Mineral oils are used as insecticides and ovicides to control plant pests and ectoparasites such as lice, ticks and fleas (Beattie, 2005).

As noted above, the use of broad-spectrum insecticides cause many adverse effects on natural enemies (Dehghani *et al*., 2013). These effect include reduction of survival (Galvan *et al*., 2005), fecundity (Papachristos and Milonas, 2008), searching potency (Moura *et al*., 2006) and nutrition (Singh *et al*., 2004). Therefore, it is imperative to study the effects of pesticides on non-target pests and natural enemies (Stark *et al*., 2004). Development of methods and techniques used to test the side-effects of pesticides on natural enemies of pests began in Europe (Almasi *et al*., 2013). Several organizations are active in this field, but the primary focus on beneficial insects was by the International Organization for Biological Control (IOBC). The organization is active in identifying pesticides that are compatible with biological control. Multiple methods used to test the toxicity of pesticides on beneficial insects have been designed by the IOBC (Hassan *et al*., 2000).

Standard bioassay tests have been developed to evaluate both lethal and sub-lethal effects of pesticides. The organization has proposed three types of test, including laboratory, semi-field and field tests (Almasi *et al*., 2013). Satyanarayana and Murthy (1991) studied the effect of several different pesticides on various developmental stages of *C. montrouzieri*. They showed that two compounds, monocrotophos and quinalphos were the most toxic to all the developmental stages of this predator. Smith and Krischik (2000) assessed the direct effect of Carbaryl and the microbial insecticide, *Beauveria bassiana,* Balsamo-Criv, Azatin (formulated from neem), insecticidal soap and horticultural oil on *C. montrouzieri*. Carbaryl had more adverse effects on the predator compared to the other compounds, while *B. bassiana* and insecticidal soap caused mortality on predatory ladybird, but Azatin and horticultural oil had very little adverse effects on adult *C. montrouzieri*. Khani *et al*., (2012) studied the adverse effects of two insecticides, imidacloprid and abamectin on adults of *C. montrouzieri* and showed that imidacloprid had higher toxicity for both male and female ladybirds compared to Abamectin.

Despite the potential impact of mineral oil along with natural predators to control citrus pests, broad-spectrum pesticides are still widely used in northern Iran (Damavandian, 2003). Thus resulting in the disruption of natural enemies and outbreaks of pests such as citrus cushion, *P. aurantii* (Damavandian, 2007). Because of the adverse effects of pesticides and the impact of mineral oil, identifying compounds that have the minimum negative impact on the natural enemies of pests is necessary to improve integrated pest management (IPM). In this study, the toxicity of three insecticides, Diazinon, Malathion, Chlorpyrifos and different concentrations of mineral oil were tested on the third and fourth larval stages and adults of *C. montrouzieri* under laboratory conditions to provide a background for implementation of integrated management programs.

Materials and Methods

Rearing

Colony of citrus mealybug, *Planococcus citri* Risso was obtained from Amol Biological Control Research Laboratory (Iranian Research Institute of Plant Protection). Budded potato tubers were used as laboratory host to maintain the citrus mealybug colony. Potato tubers (*Solanum tuberosum* Linnaeus) were thoroughly washed and placed for 25 to 30 days in a dark chamber to bud. Then, the citrus mealybug adults were released on the buds. Initial colonies of *C. montrouzieri* (50 pairs) were obtained from an insectary in Noor city. It should be noted that the emerged adults were visually sexed by examination of the first pairs of legs; as in males these legs are reddish brown to yellow while in females they are black (Pang and Gordon, 1986).A pair of the ladybirds was placed in each of 25 plastic Petri dishes with diameter and height of 9 and 2 cm, respectively. Ovisacs and different nymphal stages of citrus mealybugs reared on potatoes were used to feed these ladybirds. After 48 h, the ladybirds were removed from the Petri dishes and the laid eggs in the Petri dishes were separated. Eggs were inspected every day and after hatching (about 4 to 5 days), larvae were removed with a soft brush. Because of cannibalism, one larva was placed in each Petri dish and fed on the prey. Throughout the experiment, prey was replaced daily. To get adults, the larvae were allowed to develop and pupate. Then, pupae were transferred individually to Petri dishes. After adult emergence, sex was determined by examining the first pairs of legs; in males they are reddish brown to yellow while in females they range from grey to black (Khani *et al*., 2012). For bioassay, 3^{rd} and 4^{th} instar larvae, males and females of *C. montrouzieri* were used separately.

Bioassay experiments

In this study the organophosphate insecticides, Diazinon EC60% (Gorgan Golsam, Iran), chlorpyrifos EC40.8% (Ariashimi, Iran), and Malathion EC57% (SabzAvar Pardis, Iran) as well as mineral oil EC (Farad, Iran) (degree of sulphonation; 92%) were used, because these compounds are nowadays applied to control citrus pests. First instar larvae were placed into Petri dishes (9 cm diameter), and were fed on the mealybugs. When they reached the third and fourth instar, they were used in bioassay tests. Pupae of *C. montrouzieri*, were placed in Petri dishes (9 cm diameter). When the adults emerged, males and females were tested separately. Concentrations of the used insecticides in this test were 2000 ppm for Diazinon, Chlorpyrifos and Malathion and 10000 and 15000 ppm for mineral oil. It should be noted that these concentrations are recommended by the experts to control pests of citrus in Mazandaran province (north of Iran) (Talebi Jahromi, 2011). These experiments were conducted in four replications and fifteen individuals were tested in each replication. The Petri dishes were transferred to a potter tower to conduct spray. Delivery rate of each treatment was 1.5 ± 0.01 ml/cm^2 at 5 PSI pressure. Treated Petri dishes were allowed to dry for 1 h. After the drying, mealybugs were added into the Petri dishes to feed larvae and transferred to growth chamber at 26 ± 1 °C, 80 ± 5% RH and a photoperiod 16:8 h (L: D). Mortality was determined 24 h after treatment. Also, for each of the treatment controls only distilled water was used.

Determination of LC_{50} and LC_{90} of insecticides

To determine LC_{50} and LC_{90} of each insecticide on both male and female of *C. montrouzieri*, first, a series of preliminary tests were conducted to determine the limit of concentrations. Concentrations used in the final test were 100, 300, 500, 700 and 900 ppm for Diazinon and Malathion, 2000, 2300, 2600, 3000 and 3500 ppm for Chlorpyrifos and 15000, 18000, 21000, 25000 and 29000 ppm for mineral oil. Distilled water was used as a solvent. Fifteen ladybirds were subjected to each treatment. Each Petri dish contained five ladybirds. These Petri dishes were transferred to the Potter tower to conduct spray. The sprayed volume from each treatment was 1.5 ± 0.01 ml/cm^2 at 5 PSI pressure. Control plates were treated with distilled water. Four replications were considered for each treatment.

Compounds were classified according to IOBC standards. Compounds with a mortality lower than 30% were classified in the first category (harmless), with a mortality between 30 to 79% were classified in the second category (slightly harmful), between 80 and 99%-the third category (moderately harmful) and Compounds with a mortality more than 99% were classified in the fourth category (harmful) (Stark *et al.*, 2004).

Statistical analysis

To determine the LC_{50} and LC_{90}, probit method was used and this method was performed by the P/ PROBAN program (Van Ark, 1983). In addition, if any mortality was observed in the control treatment, other treatments' mortality was corrected with Abbott formula (Finney, 1971).

Results

Bioassay experiments

Results of tests conducted on various developmental stages of *C. montrouzieri* is presented in Table 1. Mortalities of the treated ladybirds at the recommended concentration of the studied compounds were significantly different ($F = 157.85$, $df = 19, 60$; $p < 0.01$). The results showed that the highest mortality (100%) was caused by the two insecticides, Diazinon and Malathion 24 h after treatment. The lowest mortality in L3, L4 and male adult corresponded to a concentration of 10000 ppm of mineral oil. For mineral oil, the mortality was increased as concentration increased from 10000 to 15000 ppm. Also, the minimum mortality in female occurred at concentration of 2000 ppm of Chlorpyrifos. It should be noted that females had lower sensitivity to Chlorpyrifos than mineral oil. For both Chlorpyrifos and mineral oil, adult female tended to suffer higher mortality when compared with males. Based on IOBC classification, the insecticides Diazinon and Malathion are classified as category 4 (harmful) and Chlorpyrifos and mineral oil used in both concentrations are classified as category 1 (harmless).

Table 1 Mortality rate (± SE) of different developmental stages of *Cryptolaemus montrouzieri* treated with the recommended dose of insecticides and mineral oil, and their ranking based on IOBC classification.

Compound	Developmental stage	%mortality (Mean ± SE)	IOBC ranking[1]
Diazinon (2000 ppm)	3th instar larvae	100	4
	4th instar larvae	100	4
	Adult males	100	4
	Adult females	100	4
Malathion (2000 ppm)	3th instar larvae	100	4
	4th instar larvae	100	4
	Adult males	100	4
	Adult females	100	4
Clorpyrifos (2000 ppm)	3th instar larvae	15.11 ± 4.93	1
	4th instar larvae	13.96 ± 2.66	1
	Adult males	3.57 ± 2.06	1
	Adult females	3.70 ± 2.14	1
Mineral oil (10000 ppm)	3th instar larvae	5.11 ± 3.21	1
	4th instar larvae	3.84 ± 3.84	1
	Adult males	3.57 ± 2.06	1
	Adult females	7.30 ± 3.15	1
Mineral oil (15000 ppm)	3th instar larvae	10.11 ± 4.27	1
	4th instar larvae	10.63 ± 2.19	1
	Adult males	5.23 ± 1.74	1
	Adult females	5.63 ± 3.66	1

[1] IOBC ranking of pesticides (1= harmless, 4= harmful).

Determination of LC_{50} and LC_{90} of insecticides

The bioassay results of insecticides and mineral oil on adult ladybird, *C. montrouzieri,* are shown in Table 2. The lowest mortality of males and females by the insecticide Chlorpyrifos was caused at concentrations of 2300 and 2600 ppm, respectively and the highest mortality in both males and females occurred at 3500 ppm. Comparison of mortality caused by different concentrations of this insecticide on the male and female adults of *C. montrouzieri* showed that mortality in male and female adults increases by increasing the concentration. However, mortality caused by different concentrations of this insecticide did not differ significantly ($P \geq 0.05$). Bioassay results of Diazinon showed that mortality increased in both sexes with increasing concentration, except for concentration of 300 ppm. At the highest concentration (900 ppm), the females of *C. montrouzieri* were more sensitive to the insecticide than the males. Comparison of the mean mortality caused by different concentrations showed that the mortality did not differ significantly ($P \geq 0.05$).

As expected, the insecticide Malathion caused a very high mortality in both sexes (Table 2). Also, mortality of adult ladybirds increased with increased concentrations of this insecticide. Three concentrations of 500, 700 and 900 ppm gave 100% mortality in both sexes. Comparison of bioassay data showed no significant difference in sensitivity of sexes to tested concentrations of Malathion. According to the results shown in Table 2, all the concentrations of mineral oil caused low mortality in both sexes. Also, increasing the concentration, did not change mortality rate. It should be noted that the concentrations of 21000, 25000 and 29000 ppm caused only 6.67% mortality in male ladybirds. Also, the same trend was observed in females, as nearly the same mortality rates were created by the four concentrations of 15000, 18000, 21000 and 25000 ppm. Concentration of 29000 ppm, however, caused higher mortality in females compared to males.

Table 2 The average mortality rate (± SE) of *Cryptolaemus montrouzieri* adults caused by different concentrations of studied insecticides and mineral oil 24 h after treatment.

Pesticide	Concentration (ppm)	%mortality (Mean ± SE)	
		Male	Female
Diazinon	100	3.33 ± 3.33	1.67 ± 1.67
	300	0	0
	500	26.67 ± 11.55	36.67 ± 11.39
	700	61.67 ± 9.95	58.33 ± 13.83
	900	61.67 ± 13.43	71.67 ± 11.01
Malathion	100	58.33 ± 20.81	36.67 ± 9.99
	300	92.85 ± 7.14	93.33 ± 2.72
	500	100	100
	700	100	100
	900	100	100
Chlorpyrifos	2000	0	0
	2300	0	3.34 ± 1.92
	2600	6.67 ± 2.72	1.67 ± 1.67
	3000	14.99 ± 6.87	3.34 ± 1.92
	3500	25.00 ± 11.01	21.67 ± 5.24
Mineral oil	15000	5.00 ± 3.19	6.67 ± 4.71
	18000	5.00 ± 3.19	6.67 ± 4.71
	21000	6.67 ± 6.67	6.67 ± 1.67
	25000	6.67 ± 2.72	6.67 ± 2.72
	29000	6.67 ± 2.72	20.00 ± 7.85

Probit analysis results and the LC_{50} and LC_{90} values of insecticides for adults of *C. montrouzieri* are presented in Table 3. According to the results, the males were more sensitive than the females to Chlorpyrifos. While, for insecticide Diazinon, the females were more sensitive than males to both the lethal concentrations. Based on the LC_{50} and LC_{90} values 24hours after treatment, the males and females of *C. montrouzieri* are more sensitive to Diazinon compared with Chlorpyrifos. It should be noted that due to the high mortality caused at low concentrations of Malathion and the low mortality caused by mineral oil even at high concentrations, calculating LC_{50} and LC_{90} of these compounds was not feasible. The test for fit of line (parallelism) by P/ PROBAN software showed that the hypothesis of parallelism was not rejected and the lines had slopes that were not significantly different (X^2 = 5.42, df = 3, tail p = 0.142, p > 0.05).

Table 3 The probit analysis results of insecticides chlorpyrifos and diazinon on *Cryptolaemus montrouzieri* 24h after treatment.

Insecticide	Sex	n	Slope ± SE	LC_{50} (ppm) (95% CI)	LC_{90} (ppm) (95% CI)
Chlorpyrifos	Male	360	0.89 ± 0.32	4238 (3736-6981)	5683 (4606-11929)
	Female	360	1.00 ± 0.32	4316 (3847-5898)	5480 (4610-8551)
Diazinon	Male	360	2.31 ± 0.37	701 (627-794)	1257 (1091-1560)
	Female	360	2.29 ± 0.59	635 (512-718)	1194 (1012-1727)

CI: Confidence intervals.

Discussion

The use of insecticides compatible to biological control agents is a useful tool in IPM programs (Stark and Banks, 2003), because selective application of insecticides can reduce adverse effects of pesticides on natural enemies (Galvan *et al*., 2005). In this study, it was found that the two insecticides Diazinon and Malathion at proposed concentration (2000 ppm) caused 100% mortality to each of the life stages, L_3, L_4 and adults (male and female) of *C. montrouzieri*. Thus, the use of Diazinon and or Malathion causes high mortality to this natural enemy of mealybug which may result in outbreak of this pest and other pests such as scales, citrus cushion and aphids (Dalci *et al*., 2011). The higher toxicity of diazinon on natural enemies, in comparison with other compounds, has been proven in other investigations. For example, Aghabaglou *et al*., (2012) showed that Diazinon was more toxictoL$_4$ and adults of *C. montrouzieri* than Imidacloprid. Their results showed that diazinon at a concentration of 1000 ppm caused 57% mortality on female which according to IOBC classification was classified as category 3 (moderately harmful). The difference observed in the results of these researchers with those of this study is because of different concentrations studied (1000 and 2000 ppm). Diazinon, besides direct effects, also had negative effects on *C. montrouzieri* upto indirect contact. Results of the mentioned researchers showed that indirect contact of Diazinon with female of this predator has caused 75% mortality and according to the IOBC was classified as category 4 (harmful), which is in agreement with the results of this study. Aghabaglou *et al*., (2013) reported that the lethal concentration of 1000 ppm of Diazinon caused 50% mortality to the eggs of *C. montrouzieri*. Also, the percent hatching of eggs treated with sub-lethal concentration of 500 ppm of this insecticide was only 65%. Alvandy *et al*., (2013) showed that Diazinon affects developmental stages, reproductive and population growth parameters of *C. montrouzieri* and reduces the number of eggs laid and the oviposition period. James and Coyle (2001) stated that Diazinon has high toxicity on *Harmonia axyridis* Pallas and causes high mortality of 66-100%.

The results of Gabor *et al*., (2015) showed that the insecticide Malathion has caused high mortality in the population of *Coccinella undecimpunctata* Linnaeus and *Chrysoperla carnea* Stephens with an average of 96% and 77% respectively, and was classified as category 4 (harmful), which is in agreement with the results of this study. Also, Ghadamiary and Talebi Jahromi (2002), in a study conducted on the side effects of four types of pesticides on the predator *Orius albidipennis* Reuter, found that according to the IOBC, Malathion with 100% mortality would be placed in category 4 (harmful). This result is consistent with the findings of this study.

The results of tests carried out by insecticide Chlorpyrifos showed that the highest rate of mortality in response to exposure to Chlorpyrifos was recorded for the third larval instar, followed by the fourth larval instar. Adult males and females experienced low mortality when exposed to the recommended dose of Chlorpyrifos. According to our research, Chlorpyrifos had lower toxicity than the other two chemicals on larvae and adults of this ladybird which is in agreement with the results of Satanarayana and Moretti (1991). These researchers showed that the two compounds Monocrotophos and quinalphos have higher toxicity than Chlorpyrifos on all biological stages of *C. montrouzieri*. Besides direct lethal effects, this insecticide has indirect undesirable effects on natural enemies. Also, sub-lethal effects of Chlorpyrifos have been examined in various studies, and indicate that sub-lethal concentrations of Chlorpyrifos cause negative effects such as increased duration of larval and pupal periods and decreased weight of pupae. These effects were also obvious in low concentrations (a quarter of concentration recommended) (Mostafaloo *et al*., 2012a). So, when using this insecticide, in addition to lethal concentration, sub-lethal effects on natural enemies should also be noted. Pasqualini and Civolani (2003) reported that Chlorpyrifos causes mortality between 40-63% on the coccinellids *Oenopia conglobata* Linnaeus, *Adalia bipunctata* Linnaeus and *C. septempunctata*. Al-Doghairi *et al*., (2004) stated that insecticide Chlorpyrifos was highly toxic to the ladybird *Adonia variegate* Goeze at dosages of half, one and two-folds of the recommended, where percent mortality caused by this compound was between 46-55%. Thomson (2012) reported that according to the IOBC, impact of organophosphate insecticides Chlorpyrifos, Diazinon and Malathion to predatory ladybirds and bugs were classified as categories 3 (moderately harmful) and 4 (harmful), respectively, that is consistent with the results of this study.

Comparison of percent mortality caused by high concentrations of mineral oil showed that fourth instar larvae and male of *C. montrouzieri* had the highest and lowest sensitivity to this compound, respectively. Results of Ranjbar and Heidari (2010) were Similar to our results. They showed that according to the IOBC, concentration of 5000 ppm of mineral oil on 1st instar larvae and adults of ladybird *Chilocorus bipustulatus* Linnaeus was classified as category 1 (harmless). Smith and Krischik (2000) reported that insecticide Carbaryl is highly toxic to *C. montrouzieri*. Also, they stated that microbial insecticide *B. bassiana* caused mortality on this predator, but mineral oil caused far less mortality than other compounds that is in agreement with results of this study. Contreras *et al*., (2005) reported that based on IOBC classification, effect of mineral oil to *C. carnea* and *Chrysoperla externa* Hagen was classified as category 1. These results also are consistent with the findings of our study. Gardner-Gee *et al*., (2013) studied side effects of mineral oil on *C. carnea* and *C. septempunctata*. Based on the results obtained, they placed this compound in the category 1.

One of the new approaches used to control pests is integration of release of natural enemies and the use of harmless pesticides. Many information is not readily available about mixing pesticides and their effect on natural enemies, but Mostafaloo *et al*., (2012b) stated that mixing Chlorpyrifos and mineral oil to control citrus mealybug intensified the negative effects of these compounds on survival and development of ladybird. Therefore, before mixing pesticides, the side effects of such mixtures on natural enemies must be studied, because the success of the biological control in an IPM program depends mainly on the correct selection of insecticides that would be harmless to natural enemies of pests. Based on the results of this study and mineral oil being harmless on ladybird *C. montrouzieri*, it is suggested that mineral oil and release of *C. montrouzieri* can simultaneously be used to control citrus mealybug.

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ارزیابی آزمایشگاهی سمّیت روغن معدنی، دیازینون، مالاتیون و کلرپایریفوس روی کفشدوزک *Cryptolaemus montrouzieri* Mulsant (Col.: Coccinellidae)

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چکیده: سمّیت حاد غلظت‌های توصیه شده مزرعه‌ای سه حشره‌کش رایج (دیازینون، مالاتیون و کلرپایریفوس) و روغن معدنی روی سنین سوم و چهارم لاروی و حشره بالغ *Cryptolaemus montrouzieri* Mulsant مورد ارزیابی قرار گرفت. مرگ‌ومیر ایجاد شده توسط حشره‌کش‌ها و روغن معدنی به‌طور معنی‌داری متفاوت بودند. دیازینون و مالاتیون با مرگ‌ومیر ۱۰۰ درصدی بالاترین سمّیت را نسبت به مراحل مختلف رشدی کفشدوزک نشان دادند. کلرپایریفوس و روغن معدنی مرگ‌ومیر کم‌تر از ۳۰ درصد را موجب شدند، درحالی‌که روغن معدنی کم‌ترین اثر مضر را بر این شکارگر داشت. براساس مقادیر LC_{50} و LC_{90} ۲۴ ساعت پس از تیمار، حشرات بالغ نر و ماده *C. motrouzieri* به دیازینون (۷۰۱ و ۶۳۵، ۱۲۵۷ و ۱۱۹۴) نسبت به کلرپایریفوس (۴۲۳۸ و ۴۳۱۶، ۵۶۸۳ و ۵۴۸۰) حساس‌تر بودند. براساس طبقه‌بندی سازمان بین‌المللی کنترل بیولوژیکی (IOBC)، کلرپایریفوس و روغن معدنی به‌عنوان دسته یک (بی‌خطر) طبقه‌بندی شدند و دیازینون و مالاتیون در دسته چهار (خطرناک) قرار گرفتند.

واژگان کلیدی: سمّیت حاد، زیست‌سنجی، *Cryptolaemus montrouzieri*، حشره‌کش اورگانوفسفره، روغن معدنی