

#### **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  $3^{rd}$  and  $4^{th}$  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<sub>50</sub> and LC<sub>90</sub> 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; Kairoet 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).

decades, In recent 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 predatory ladybird, but Azatin and on 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 impact of mineral oil, identifying the compounds that have the minimum negative impact on the natural enemies of pests is necessary improve integrated to 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, 3rd and 4th 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  $1.5 \pm 0.01 \text{ ml/cm}^2$  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  $26 \pm 1$  °C,  $80 \pm 5\%$  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<sub>50</sub> and LC<sub>90</sub> of insecticides To determine LC<sub>50</sub> and LC<sub>90</sub> of each insecticide on both male and female of C. montrouzieri, first, a series of preliminary tests were conducted to of determine the limit 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  $\pm$  0.01 ml/cm<sup>2</sup> 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 $\pm$ SE)	IOBC ranking <sup>1</sup>
Diazinon	3 <sup>th</sup> instar larvae	100	4
(2000 ppm)	4 <sup>th</sup> instar larvae	100	4
	Adult males	100	4
	Adult females	100	4
Malathion	3 <sup>th</sup> instar larvae	100	4
(2000 ppm)	4 <sup>th</sup> instar larvae	100	4
	Adult males	100	4
	Adult females	100	4
Clorpyrifos	3 <sup>th</sup> instar larvae	$15.11 \pm 4.93$	1
(2000 ppm)	4 <sup>th</sup> instar larvae	$13.96 \pm 2.66$	1
	Adult males	$3.57 \pm 2.06$	1
	Adult females	$3.70 \pm 2.14$	1
Mineral oil	3 <sup>th</sup> instar larvae	$5.11 \pm 3.21$	1
(10000 ppm)	4 <sup>th</sup> instar larvae	$3.84 \pm 3.84$	1
	Adult males	$3.57 \pm 2.06$	1
	Adult females	$7.30 \pm 3.15$	1
Mineral oil (15000 ppm)	3 <sup>th</sup> instar larvae	$10.11 \pm 4.27$	1
	4 <sup>th</sup> instar larvae	$10.63 \pm 2.19$	1
	Adult males	$5.23 \pm 1.74$	1
	Adult females	$5.63 \pm 3.66$	1

<sup>1</sup>IOBC ranking of pesticides (1= harmless, 4= harmful).

Determination of LC<sub>50</sub> and LC<sub>90</sub> 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  $\ge$  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  $(\pm SE)$  of *Cryptolaemus montrouzieri* adults caused by different concentrations of studied insecticides and mineral oil 24 h after treatment.

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

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<sub>50</sub> and LC<sub>90</sub> 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 $\pm$ SE	LC <sub>50</sub> (ppm) (95% CI)	LC <sub>90</sub> (ppm) (95% CI)
Chlorpyrifos	Male	360	$0.89 \pm 0.32$	4238	5683
				(3736-6981)	(4606-11929)
	Female	360	$1.00 \pm 0.32$	4316	5480
				(3847-5898)	(4610-8551)
Diazinon	Male	360	$2.31 \pm 0.37$	701	1257
				(627-794)	(1091-1560)
	Female	360	$2.29 \pm 0.59$	635	1194
				(512-718)	(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<sub>3</sub>, L<sub>4</sub> 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<sub>4</sub> 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 Satanaravana 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 Cseptempunctata. 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 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 حشره کش رایج (دیازینون، مالاتیون و کلرپایریفوس) و روغن معدنی روی سنین سوم و چهارم لاروی و حشره بالغ Cryptolaemus معدنی بهطور معنیداری متفاوت بودند. دیازینون و مالاتیون با مرگومیر ۱۰۰ درصدی بالاترین سمّیت 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 کم تر از ۳۰ درصد را موجب شدند، درحالی که روغن معدنی کم ترین اثر مضر را بر این شکار گر داشت. براساس مقادیر 200 و 10% معایت پس از تیمار، حشرات بالغ نر و ماده Controuzieri به دیازینون (۷۰۱ و ۲۶۵، ۱۵۹۷ و ۱۱۹۴) نسبت به کلرپایریفوس (۲۳۸۴ و ۲۳۹۶، ۵۶۸۳ و ۵۴۸۰) حساس تر بودند. براساس طبقهبندی سازمان بینالمللی کنترل بیولوژیکی (IOBC)، کلرپایریفوس و روغن معدنی به عنوان دسته یک (بی خطر) طبقهبندی شدند و دیازینون و مالاتیون در دسته چهار (خطرناک) قرار گرفتند.

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