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Lethal and side effects of the acaricides spirodiclofen and spiromesifen on the two-spotted spider mite, *Tetranychus urticae* Koch, and its predatory mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)

S. Sarbaz^{1*}, Sh. Goldasteh², A. A. Zamani³, E. Soleyman-Nejadian⁴, R. Vafaei Shoushtari²

Ph.D Student, Department of Plant Protection, Faculty of Agriculture, Islamic Azad University, Arak Branch, Arak, Iran
Assistant Professor, Department of Plant Protection, Faculty of Agriculture, Islamic Azad University, Arak Branch Arak, Iran

3- Associate Professor, Department of Plant Protection, Faculty of Agriculture, Razi University, Kermanshah, Iran

4- Associate Professor, Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University, Ahvaz, Iran

5- Assistant Professor, Department of Plant Protection, Faculty of Agriculture, Islamic Azad University, Arak Branch Arak, Iran

Abstract

The acute toxicity of the acaricides spirodiclofen and spiromesifen to eggs and adult females of the two-spotted spider mite, *Tetranychus urticae* Koch, was evaluated in laboratory at $27 \pm 2^{\circ}$ C temperature, $70 \pm 5\%$ relative humidity (RH), and 16/8 L/D photoperiodic conditions. The residual effects of these miticides on adult females were also assessed. In these assays, eggs and female mites were treated with five different concentrations of the two acaricides: spirodiclofen (0.21, 0.41, 0.83, 1.63, and 3.26 mg/L) and (0.79, 3.78, 18.11, 86.87, and 416 mg/L), spiromesifen (0.02, 0.06, 0.18, 0.53, and 1.56 mg/L) (0.39, 4.07, 10.10, 51.27, and 260 mg/L) for egg and female adult respectively. LC_{50} and LC_{90} of the chemicals were measured and the obtained data was analysed by probit analysis. The LC_{50} value of spirodiclofen against egg and adult of *T.urticae* were 0.86, 11.95 and for spiromesifen were 0.10, 5.95 ppm respectively. Also the effects of the two acaricide residues on mortality rate of female spider mites and its predatory mite, Neoseiulus californicus McGregor, were investigated at LC₉₀ level following 0, 1, 3, 7, 10 and 14 days' of treatment. at The results showed that the mortality of mites increased significantly with increasing acaricide concentrations. Generally, both acaricides, especially spiromesifen, proved to be highly toxic to eggs, moderately to adult females, and slightly to predatory female mites. After seven days of treatment, mortality in mites caused by spirodiclofen and spiromesifen were 86.6% and 70%, and after two weeks, were 46.6% and 51.6%, respectively. After two weeks of treatment, acaricides had gained efficient control against two-spotted spider mites and did not have a significant negative effect on the population of N. californicus. The results suggest that both spirodiclofen and spiromesifen could be successfully used for an IPM programme acco pany with the biological control agent, N. californicus.

Keywords: Tetranychus urticae, Neoseiulus californicus, Spirodiclofen, Spiromesifen, Toxicity

^{*} Corresponding Author, E-mail: sa.sarbaz@gmail.com Received:10 June. 2017 – Accepted: 19 Aug. 2017

Introduction

Two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important agricultural pests with about 1, 200 species of host plants. Their feeding activity plays an important role in many cropping systems worldwide. Recently, outbreaks of *T. urticae* significantly reduced the yield of leguminous plants. Legumes are economically important crops, commercially grown on more than 180000 ha annually in Iran (Motazedian *et al.*, 2012).

When mite density is at or above the economic threshold level, chemical control should be applied frequently to save the crop. Among the synthetic acaricides, the tetronic acid derivatives spirodiclofen and spiromesifen were reported to be promising candidates for integrated pest management programmes in leguminous plants. Because of their novel mode of action (inhibition of lipid synthesis), these compounds effectively control mite populations that are resistant to other acaricides. In baseline susceptibility bioassays with two-spotted spider mites, spirodiclofen and spiromesifen showed excellent efficacy against all developmental stages of the species. High toxicity to eggs and immature developmental stages and slower activity against adult females with a strong negative influence on female fecundity and fertility were observed when mites are exposed to sublethal doses of the miticides (Nauen *et al.*, 2005; Marcic *et al.*, 2010; Yu *et al.*, 2011).

However, the application of miticides may not always be effective due to the rapid evolution of miticide-specific resistance in the mite populations (Maciesiak & Olszak 2006; Marcic *et al.*, 2009; Yu *et al.*, 2011). Thus, using the combination of acaricides and phytoseiid mites has been suggested to be the most effective management strategy for this pest. In fact, successful biological control of *T. urticae* can be achieved through the compatibility of predators with current pesticides used in targeted agricultural systems. *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) is an important widespread predatory mite that has been successfully used to control spider mites in horticultural crops grown in the field and in greenhouses in Iran (Maroufpoor *et al.*, 2016).

Worldwide, a large volume of information is available regarding the effect of spirodiclofen and spiromesifen on two-spotted spider mites and phytoseiid mites. However, in Iran, this information is limited (Ibrahim & Yee, 2000, Maciesiak & Olszak, 2006; Marcic 2007; Marcic *et al.*, 2009; Hamedi *et al.*, 2010; Park *et al.*, 2011; Al-Lala *et al.*, 2012; Alinejad *et al.*, 2016; Maroufpoor *et al.*, 2016). Marcic *et al.*, (2011) reported that spirodiclofen produced some adverse effects on predatory mites, even when applied at the recommended rates. *N. californicus* shows high resistance to fenpyroximate, fenpropathrin, dimethoate, propargite, sulphur, and benomyl compared to *T. urticae*, as observed in the mite populations collected in strawberry fields in Brazil (Sato *et al.*, 2002).

For ensuring a successful integrated pest management programme, it is necessary to evaluate the overal impact of acaricides on the mortality, fecundity, fertility immature stages and of pests and predatory mites. which can be achieved through bioassays. The current study is designed to evaluate the efficacy of spirodiclofen and spiromesifen toxicity to eggs and adult females of *T. urticae*, as well as to assess the side effects on the predator, *N. californicus*.

Material and Methods

Growing host plants

Bean seedlings of *Phaseoulus vulgaris* L. var. Akhtar which is one of the important hosts of this mite were used for rearing and toxicological tests of the two-spotted mites. Seeds were sown directly in pots with 3 cm diameter, filled with soil, peat moss, and sand in a 2:1:1 ratio respectively. The plants were infested with *T. urticae* when they reached the true leaf stage. No pesticides were applied on the plants and maintained under greenhouse conditions at 35 ± 2 °C temperature, $60 \pm 5\%$ relative humidity (RH), and a photoperiod of 16:8h (L:D).

Population tested

The colonies of *T. urticae* were collected from infested bean in the field in Arak regian (Iran) and reared on detached bean leaves. The detached leaves of kidney bean were maintained on moistened cotton in Petri dishes (15 cm in diameter) surrounded by saturated cotton to prevent escaping of the mites. The rearing arena was covered with a ventilated lid. The colonies of *N. californicus* were obtained from Giah Bazr Alvand Company, an agent of Koppert Company in Tehran. They were reared in the laboratory on bean leaves infested with *T. urticae*. The stock culture of two-spotted mites and the predatory mite were kept in growth chamber at 27 ± 2 °C, $70 \pm 5\%$ relative humidity (RH), and photoperiod of 16:8 h (L: D).

Chemicals tested

Spirodiclofen (commercial formulation name Envidor® suspension concentrate, 240 g a.i./l, Bayer Crop Science, Germany) and spiromesifen (commercial formulation name Oberon® suspension concentrate, 240 g a.i./l, Bayer Crop Science, Germany) were obtained from Giah Bazr Alvand Company, Iran.

Toxicity bioassays

The toxicity of two pesticides was measured using a detached bean leaf dip bioassay method similar to that described by Yamamoto *et al.*, (1995) and Hu *et al.*, (2010). Freshly cut bean leaves were dipped for 8 sec in 5 different concentrations of acaricides, which were prepared by using distilled water containing 1 g/L of the nonionic surfactant Triton X-100, and then allowed to air dry for 30 minutes. Leaves treated with distilled water containing Triton X-100 alone were used as control. When the leaves dried, discs of approximately 3 cm diameter were cut from the dry leaf and placed in Petri dishes (9 cm in diameter) upside down. After 72h, the mite mortality was assessed. Female adult mites were considered dead if their appendages did not move within 5s of being touched with a camel-hair brush.

For the egg bioassays, 20 female spider mites were released on untreated bean leaves placed upside down on a water-soaked cotton pad in a Petri dish. The females were allowed to oviposit for 24h and then females removed. Each leaf with 20 eggs was dipped in an acaricide solution for 8 sec. Then, the leaves were checked daily to record the number of eggs hatching. Pesticides concentrations ranged from 0.79-416 mg/L to 0.21-3.26 mg/L for spirdiclfen and 0.39- 260 mg/L to 0.02-1.56 mg/L for spiromesifen against adults and eggs, respectively. The range of concentrations was chosen on the basis of preliminary trials. Leaves treated with distilled water containing Triton X-100 alone were used as control. Each bioassay was repeated five times. The Petri dishes were maintained in growth chamber conditions $(27 \pm 2^{\circ}C; 70 \pm 5\% \text{ RH}; 16:8 h (L: D)).$

Residual effects of acaricides on adult females of spider mites and predatory mites

Ten young bean plants with 6–8 leaves were selected for each treatment. The commercial products were applied using a trigger-operated hand sprayer, at the LC_{90} value (72h) rates: spirodiclofen 190.63 mg/L (Envidor SC, 24% Bayer CropScience) and spiromesifen, 451.25 mg/L (Oberon SC, 24%; Bayer CropScience), until run-off. A group of ten plants was treated with water as control. The plants were allowed to dry and placed in a plastic greenhouse. Twenty same-aged (0-24h-old) adult female mites were transferred on the treated and control leaf discs with a fine soft-pointed brush 0, 1, 3, 7, 10 and 14 days after spraying. The experiment for assessing the residual effects was conducted with five replicates per treatments (spiromesifen, spirodiclofen and water), with 15 females per replication, were used in each time period. The leaves were checked daily to record the mortality and survival rates of female mites until the death. Mortality was assessed after 24 h of exposure.

For predatory mites the same method (Leaf-Spray method) was applied and 15 females were used in these treatments. *T. urticae* as prey was used on leaf after spraying. Mortality was recorded after 72 h of applying.

Statistical analysis

Concentration-mortality data was subjected to probit analysis (Finney, 1971) using a Maximum Likelihood Program (POLO-PC, LeOra Software, Berkeley, California) to determine the LC_{50} and LC_{90} (and other LCs if required) values, their 95% confidence limits, slope and intercept of probit mortality regressions, and the relevant statistical tests (such as "t" ratio, 'g' factor and heterogeneity). For comparison of the probit mortality lines of treatments, the program also provides the likelihood ratio tests of equality and parallelism (Russel *et al.*, 1997). The resistance ratio and 95% confidence limits of this ratio were determined between data from different oil treatments, with comparisons made as described by Robertson & Preisler (1992). The estimates of parameters needed for computing confidence limits of the resistance ratio were provided by individual probit analysis in the POLO-PC output. The persistence data (i.e. percentage mortality of the adults every 72 h from the start) were transformed to arcsine square-root before performing three-way full factorial analysis of variance (ANOVA). The mean comparisons were made using the Duncan multiple range test. Means of untransformed data were reported.

For residual tests, results were evaluated using analysis of variance to determine the effects of pesticides on the two spotted spider mite and predatory mites. The Tukey's test, a multiple comparison method, was used to evaluate the differences between group averages. The corrected mortality of the three pesticide applications was determined, according to Abbott (1925). The results of predatory mites were classified according to Hassan (1992) and Sterk *et al.* (1999) as follows: harmless (0–30% effect), slightly harmful (30–79% effect), moderately harmful (80–99% effect) and harmful (99–100% effect).

Results

Lethal effects of acaricides on egg and adult females of the spider mites

The results obtained from the toxicity bioassay are shown in Table 1. Comparisons among the toxicity of two pesticides were obtained using probit analysis. The dose-mortality responses of *T.urticae* females to the pesticides were compared in terms of differences in slope and/or intercept of probit regressions and the LC₅₀ values (Table 1). The results for pesticide efficacy of each dose showed that mortality increased with inceare in the pesticides concentrations. The heterogeneity factors of all bioassays were less than 1, that showed there was no sign of systematic deviations in the chi-square (χ 2) values. For two pesticides, the regression tests ("t" ratio) were greater than 1.96 and the potency estimation tests ("g" factor) were less than 0.5 at all probability levels (Table 1). The slopes of probit morality regressions for two pesticides against egg and female were significantly different, as revealed by rejection of the likelihood ratio test of parallelism (χ 2=69.185, df=3, *P*<0.001). Further likelihood ratio tests on eggs revealed that the slope of probit mortality regression for spirodiclofen was significantly greater than the corresponding slopes of spiromesifen (χ 2=15.31, df=1, *P*<0.001). Similar comparisons on adults showed that the slope of probit mortality regression for spirodiclofen was significantly greater than spiromsifen (χ 2=11.69, df=1, *P*<0.001). (Table 1).

On the basis of likelihood ratio tests, there was significant difference between the slopes of egg and female probit mortality regressions for spirodiclofen ($\chi 2=31.98$, df=1, P<0.001), spiromsifen ($\chi 2=8.26$, df=1, P<0.001) (Table 1). The intercepts of probit mortality regressions for two pesticide were significantly different, as revealed by rejection of the likelihood ratio test of equality ($\chi 2=373.58$, df=6, P<0.001). Further likelihood ratio tests of equality indicated that the intercepts between all possible paired combinations differed significantly from each other (P<0.001) (Table 1). The above differences in slopes and/or intercepts of the probit mortality regressions among experimental treatments were

The toxicity data with the LC_{50} values and their respective 95% confidence limits indicated that spirodiclofen had the highest contact toxicity to both stages, while spiromsifen had the lowest contact toxicity and spiromesifen with lower LC_{50} was more harmful to eggs and adult females than spirodiclofen (Table 2). Comparisons between susceptibility of egg and female to each pesticide indicated that egg were more susceptible than females to pesticides (Table 3). Compared to the LC_{90} results, spirodiclofen with lower LC_{90} proved to be more toxic than spiromesifen to the growth stages of *T. urticae* (Table 4).

At the LC_{90} level, however, no significant differences between the two acaricides were detected in the susceptibility of treated females (Table 5.)

Pesticide	Growth stage	n -	Probit mortality concentration		<i>....</i> ,	Hetero	g (0.95	Lethal concentrations (mg/L) (95% confidence limits)	
			Slope (±SE)	Intercept (±SE)	<i>'t</i> '' ratio	Heterogeneity	(0.95) factor	LC ₅₀	LC ₉₀
Spirodiclofen	Egg	600	2.20±1.18	-0.69±0.1	11.08	0.35	0.03	0.86 0.72-1	3.29 2.61-4.45
	Adult	600	1.07±0.96	-1.55±0.19	11.10	0.03	0.03	11.95 8.10-16.90	190.63 121.7- 341.70
Spiromesifen	Egg	600	1.04±0.11	-64± .81110281E- 01	9.72	0.81	0.04	0.1 0.07-0.14	1.70 1.10-3.31
	Adult	600	0.69±0.06	-0.81±0.11	11.23	0.72	0.03	6.24 3.85-9.69	451.25 217.26- 1258.23

Table 1- Probit analysis of spirodiclofen and spiromesifen toxicity after the treatment of eggs and adult females
of the species Tetranychus urticae

n: Total number of test insects including control

Table 2- Comparison of the susceptibility of different growth stages of T. urticae to the toxicity of spirodiclofen and spiromesifen
according to LC_{50} ratios and their confidence limits as calculated ^a

Variable	Ratio LC ₅₀	(95% confidence limit) ^a
Pesticide	Adult (LC ₅₀) / Egg (LC ₅₀)	Comparing different growth stages
Spirodiclofen	13.90	13.56-14.23*
Spiromesifen	61.71	$60.37-63.08^{*}$

^{a:} Lower and upper 95% confidence limits calculated as described by Robertson and Preisler (1992).

*: Significant difference at P < 0.05.

Variable	Ratio LC ₅₀	(95% confidence limit) ^a	
Growth stages	LC ₅₀ /LC ₅₀ (Spiromesifen) (Spirodiclofen)	Comparison of two pesticides	
Egg	0.96	0.66-1.38*	
Adult	1.96	$1.06-3.46^{*}$	

Table 3- Comparison of the toxicity of spirodiclofen and spiromesifen on mortality of different growth stages of T. urticae, according to LC₅₀ ratios and their calculated confidence limits^a

^a: Lower and upper 95% confidence limits calculated as described by Robertson and Preisler (1992).

Table 4. Comparison of the susceptibility of different growth stages of T. urticae to the toxicity of spirodiclofen and spiromesifen according to LC90 ratios and their confidence limits calculated^a

Variable	Ratio LC ₉₀	(95% confidence limit) ^a
Pesticide	Adult (LC ₉₀) / Egg (LC ₉₀)	Comparing different growth stages
Spirodiclofen	0.42	$0.24 - 0.75^*$
Spiromesifen	264.4	94.35-740.9 [*]

: Lower and upper 95% confidence limits calculated as described by Robertson and Preisler (1992).

*: Significant difference at P < 0.05.

Table 5- Comparison of the toxicity of spirodiclofen and spiromesifen on mortality of different growth stages of T. urticae, according to L C₁₀ ratios and their confidence limits calculated^a

Variable	Ratio LC ₉₀	(95% confidence limit) ^a
Growth stages	/ LC ₉₀ (Spiromesifen) LC ₉₀ (Spirodiclofen)	Comparing two pesticides
Egg	1.93	1.04-3.59*
Adult	0.42	0.15-1.16 ^{ns}

calculated as described by Robertson and Preisler (199

*: Significant difference at P < 0.05.

ns: No significant differences at P < 0.05.

Residual effects of acaricides on mortality of adult females of the spider mite and predatory mite

The results of residual effect showed that there was a significant difference between the mortality of female spider mites caused by two acaricides on the first and third days of treatment (Table 6). On the seventh day after the treatment, spiromesifen appears to be highly toxic with more than 60% mortality rate in females and, after two weeks, each of the two acaricides caused an approximately 50% of mortality rate in females (Table 6).

Table 6- Percentage mortality (± SE) of <i>T.urticae</i> females when exposed to pesticides. Mortality %±SE of female spider mites after treatment (days)			
Days	Spiromesifen	Spirodiclofen	
0 day	90±3.33ª	91.67±3.19 ^a	
1 th day	86.67 ± 2.72^{a}	70 ± 6.93^{b}	
3 th day	78.33±7.39 ^a	56.67±6.38 ^b	
7 th day	68.33±11.01 ^a	61.67 ± 3.19^{a}	
10^{th} day	53.33 ± 2.72^{a}	51.67±4.19 ^a	
14 th day	51.66 ± 3.19^{a}	46.67 ± 6.08^{a}	

Mean entries within a row followed by the same letter are not significantly different (Tukey: $\alpha = 0.05$)

In contrast, the two acaricides (at LC₉₀ level) did not show any significant different on the mortality of females of predatory mites during treatment except on the third day (Table 7). On the first day, spirodiclofen and spiromesifen caused 51.66% and 50% mortality, respectively. When the toxic effects of the acaricides. According to the classification of Hassan (1992), they are listed as slightly harmful (class 2, 30-79). Spirodiclofen and spiromesifen were harmless on the seventh (15% mortality and reflected in the LC₉₀ or LC₅₀ estimates.

11.66 %), 10^{th} (3.33% and 1.67%), and 14^{th} (1.67% and 0%) days, respectively (Table 7). Both acaricides presented no toxic effect on *N. californicus* after 10 days.

Mortality %±SE of female spider mites after treatment (days)				
Days	Spiromesifen	Spirodiclofen		
0 day	50 ± 1.92^{a}	51.66±3.19 ^a		
1 th day	35 ± 1.66^{a}	37 ± 1.22^{a}		
3 th day	25 ± 1.66^{b}	31.66 ± 3.19^{a}		
7 th day	11.66 ± 1.92^{a}	15 ± 1.66^{a}		
10 th day	$1.67{\pm}1.67^{\rm a}$	3.33 ± 1.92^{a}		
14 th day	1.67 ± 1.67^{a}	1.67 ± 1.67^{a}		

Table 7- Percentage mortality (± SE) of Neoseiulus californicus females when exposed to pesticides

Mean entries within a row followed by the same letter are not significantly different (Tukey: $\alpha = 0.05$)

Discussion

The bioassay results of spirodiclofen and spiromesifen on the growth stages of two-spotted spider mites indicated that both miticides were highly toxic to eggs and females, spiromesifen was more toxic to the developmental stages than spirodiclofen. The same trends were observed by Yu *et al.*, (2011), who argued that *Panonychus citri* McGregor developed a slower resistance to spirodiclofen compared to other acaricides when selected continuously in increasing concentrations under laboratory conditions.

A similar pattern of toxicity against the two-spotted spider mite has been previously reported for spirodiclofen (Marcic 2007; Van Pottelberge *et al.*, 2009) and spiromesifen (Nauen *et al.*, 2005; Marcic *et al.*, 2010). In several European countries, spirodiclofen was found to provide a good protection against *T. urticae* when applied at a 96 mg/l concentration (Elbert *et al.*, 2005). Al-Lala *et al.*, (2012) reported that all the tested field populations of *T. urticae* were relatively resistant to spiromesifen. Marcic *et al.*, (2009) explained that spiromesifen affected fecundity, fertility, and population growth rates of the *T. urticae* females. They concluded that the females treated with 180 ppm of spiromesifen laid no eggs and most died within a few days after treatment. Van Pottelberge *et al.*, (2009) reported cross-resistance between spirodiclofen and spiromesifen in a laboratory-selected strain of *T. urticae*. The existence of cross-resistance among tetronic acid derivative acaricides potentially limits their performance.

Based on the LC₅₀ and LC₉₀ levels, eggs and adult females are respectively susceptible and resistant stages to acaricides, especially to spiromesifen. It was previously documented by Marcic *et al.*, (2007, 2009, 2010) that sublethal doses of spirodiclofen and spiromesifen showed high toxicity to eggs and immature stages, and slower activity against female adults. However, a strong negative influence on female fecundity and fertility was also observed. Yu *et al.*, (2011) suggested that the eggs of *P. citri* collected from fields showed high resistance to spirodiclofen. These differences may be caused by the different environmental selection and diverse resistance mechanisms of the mite species, resulting in differential sensitivity.

The residual results also indicated that the toxicity of spirodiclofen and spiromesifen persisted for 10 days with 50% mortality against eggs and adult females. Nauen *et al.*, (2005) found that the fecundity of two-spotted spider mite directly treated on bean leaves was strongly reduced 48h after treatment with spiromesifen concentration ranging from 0.064–40 mg/l. Al-Lala *et al.*, (2012) emphasized that the toxic activity on spider mite females persisted beyond nine days when they were applied at the highest recommended field rate. According to the findings of Maciesiak and Olszak (2006), spirodiclofen as well as mibemectin showed very high and long-lasting efficacy in controlling mites on apple and plum trees and, during 6-12 weeks following the treatment; the mite population did not exceed the economic threshold level.

The side effects of acaricides on predatory mites caused 51.1% and 50% mortality after 24h, when spirodiclofen and spiromesifen were applied at the recommended field rate and twice the field rate, respectively. Per IOBC classification, these miticides were slightly harmful to *N. californicus*. Rhodes *et al.*, (2006) showed that the field rate of spirodiclofen was very toxic (82-96% mortality) after seven and 21 days following treatment for *T. urticae* but not for the predatory mite, *Amblyseius andersoni* Chant. They also suggested that release of phytoseiid mites after applying bifenazate at half the recommended rate effectively controlled *T. urticae* in strawberries. Cheon *et al.*, (2007) suggested that acaricides at reduced rates might be used to adjust the predator/prey ratio. This low-concentration strategy with biological control agents within an IPM system can help to reduce selective pressure and the development of resistance (Roush 1989; Dent 2000). Acaricide treatments at rates/concentrations below the recommended level could achieve efficient control of the pest, in conjunction with the release of phytoseiid mites. Therefore, the development of field experience is essential to obtain more information about the effects of this natural active component on populations of biocontrol agents and its relationship with the probable revitalization of pest species populations.

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فصلنامه تخصصي تحقيقات حشرهشناسي

(علمي- پژوهشي)

جلد ٩، شماره ٢، سال ١٣٩۶، (١-١١)

اثرات کشندگی و جانبی کنه کش اسپیرودیکلوفن و اسپیرومسیفن روی کنه تارتن دو لکهای *Tetranychus urticae* Koch و شکارگر آن McGregor (Acari: Phytoseiidae)

سعیده سرباز'*، شیلا گلدسته'، عباسعلی زمانی'، ابراهیم سلیمان نژادیان'، رضا وفایی شوشتری'

۱– دانشجوی دکتری، گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه آزاد اسلامی اراک، واحد اراک ۲– استادیار، گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه آزاد اسلامی اراک، واحد اراک ۳– دانشیار، گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید چمران، اهواز ۴– دانشیار، گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه شهید چمران، اهواز

چکیدہ

سمیت حاد دو آفت کش اسپیرودیکلوفن و اسپیرومسیفن علیه مراحل مختلف سنی تخم و ماده بالغ کنه تارتن دولکهای (Koch) (Koch) *Tetranychus urticae* (Koch) روی گیاه لوبیا در شرایط آزمایشگاه بررسی شد. آزمایش ها در شرایط دمایی ۲±۲۷ رطوبت نسبی ۵±۷۰٪ و دوره نوری ۱۶۸ (تاریکی: روشنایی) بررسی شد. اثر باقی مانده دو آفت کش روی ماده های بالغ نیز ارزیابی شد. زیست سنجی به روش غوطه ورسازی برگ در پنج غلظت مختلف برای آفت کش اسپیرودیکلوفن (۴۱۶ ۱۸/۱۸ ۸۶/۸۷، ۲/۷۹، ۲/۷۹)، (۳/۲۶، ۱۸/۲۰، ۲۱/۰، ۲۱/۱۰) و اسپیرومسیفن (۲۶۰، ۲۰/۱۵، ۱۰/۱۰، ۲۰/۰۹، ۱۸/۱۰) ۱۸/۵۷، ۲۸/۱۰، ۲۰/۰۱، ۲۰/۰۹، ۲۰/۰۱) به ترتیب برای مرحله تخم و ماده بالغ انجام شد و سپس مقادیر محاد و موسیله آنالیز پروبیت تایید شد. مقادیر محاد باربر ۲۰/۰۹ پی پی م و ماده بالغ انجام شد و سپس مقادیر ۲۰۶۵ و ۲۰/۰۹ و ۲۸/۶۰

همچنین اثر باقیمانده دو آفتکش فوق روی مرحله کنه ماده بالغ آفت و کنه شکارگرش Neoseiulus californicus بررسی شد. اثر غلظت حاصل از موی مرحله کنه ماده بالغ آفت و میر کنه آفت و شکارگر بین روزهای اول و ۱۴ بهدست آمد. دادههای آماری حاصل از این آزمایش نشان داد که بهطور کلی هر دو آفتکش به خصوص اسپیرومسیفن سمیت بالایی روی مرگ و میر کنه ماده بالغ آفت و اثر سمیت کمی روی کنه شکارگر دارند. بعد از گذشت هفت روز تیمار شدن با دو آفتکش اسپیرومسیفن سمیت بالایی روی مرگ و میر کنه ماده بالغ آفت و اثر سمیت کمی روی کنه شکارگر دارند. بعد از گذشت مفت روز تیمار شدن با دو آفتکش اسپیرومسیفن سمیت بالایی روی مرگ و میر کنه ماده بالغ آفت و اثر سمیت کمی روی کنه شکارگر دارند. بعد از گذشت هفت روز تیمار شدن با دو آفتکش اسپیرومسیفن و اسپیرودیکلوفن مرگ و میر کنه ماده بالغ آفت به ترتیب ۹۶/۶ و ۱۸۷ مفت روز تیمار شدن با دو آفتکش اسپیرومسیفن و اسپیرودیکلوفن مرگ و میر کنه ماده بالغ آفت به ترتیب ۹۵/۶ و ۱۷۷۷ بعد از گذشت دو هفته با وجود تاثیر بالا در کنترل جمعیت کنه تارتن دو لکه ای، هیچ تاثیر منفی روی جمعیت کنه شکارگر نداشت.

واژههای کلیدی: اسپیرودیکلوفن، اسپیرومسیفن، کنه تارتن دو لکهای، Neoseiulus californicus سمیت

- * نويسنده رابط، پست الكترونيكي: sa.sarbaz@gmail.com
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