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Effect of sublethal doses of thiamethoxam and pirimicarb on functional response of *Diaeretiella rapae* (Hymenoptera: Braconidae), parasitoid of *Lipaphis erysimi* (Hemiptera: Aphididae)

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Abstract: The toxicity of thiamethoxam and pirimicarb on *Diaeretiella rapae* (Mc'Intosh) as a parasitoid of *Lipaphis erysimi* (Kaltenbach), exposed to residues on glass vials, was investigated under laboratory conditions at 23 ± 2 °C, 70 ± 5% RH and 16:8 h (L: D). Moreover, in order to find the negative impacts of both insecticides on the efficiency of *D. rapae,* the functional response of the exposed parasitoids were assessed. The newly emerged parasitoids were exposed to LC_{25} of the insecticides and distilled water as control. Host densities of 2, 4, 6, 8, 16, 32 and 64 were placed on canola seedlings in a transparent cylindrical container and were offered to the treated parasitoids. Type of functional response was determined by means of logistic regression method and the parameters, attack rate (a) and handling time (T_h) were calculated by nonlinear regression model using SAS software. The parasitoid exhibited type II functional response in all experiments. Attack rates in control, pirimicarb and thiamethoxam were 0.057 ± 0.01,0.059 ± 0.013 and 0.040 ± 0.01 h^{-1}, and handling times were 1.097 ± 0.1, 1.86 ± 0.02, 2.81 ± 0.296 h, respectively. Maximum rates of parasitism (T/T_h) were estimated 21.87, 12.9, 8.53 aphids, respectively. These observations suggest that pirimicarb with less harmful effects is the preferred candidate for controlling the mustard aphid.

Keywords: *Diaeretilla rapae*, *Lipaphis erysimi*, functional response, thiamethoxam, pirimicarb

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Introduction

Canola is one of the most important oil seeds attacked by 38 species of insect pests. The mustard aphid *Lipaphis erysimi* (Kaltenbach) is the most serious pest causing severe damages of up to 90% on canola (Bakhetia, 1987; Narang *et al*., 1993). This aphid has been found to be the dominant species in canola crops with a frequency of 66.7%, in Khuzestan province, Southwest Iran (Khajehzadeh *et al*., 2010). The yield loss caused by the mustard aphid has been raised up to 27% in Shoush city (Khajehzadeh and Kariminezhad, 2008).

Diaeretiella rapae (Mc'Intosh) is a parasitoid with a substantial role in preventing the outbreak of aphids in brassica crops

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(Bahana and Karuhize, 1986; Elliott *et al.,* 1994). This parasitoid prefers to parasitize the aphids feeding on Brassicaceae such as *L. erysimi* rather than generalist species (Blande *et al*., 2004).

Combination of biological and chemical control is the main objective of Integrated Pest Management (IPM). However, despite the presence of selective insecticides, this remains as a difficult challenge (Wennergren and Stark, 2000; Al Antary *et al*., 2010). Irregular use of pesticides in agricultural systems has unfavorable effects on non-target organisms, resulting in reduction of their efficiency in biological control. Beside the introduction of new agrochemicals, it is necessary to evaluate their potential effects on survival and efficiency of natural enemies (Paul and Thygarajan, 1992). The evaluation of these effects is more complicated than what was reported in the past (Banken and Stark, 1998).

Functional response is the main element in host- parasitoid interactions which was discussed by Solomon (1949) for the first time. It is defined as the change in number of hosts attacked by a natural enemy per unit of time in relation to host densities (Solomon, 1949; Holling, 1959; McCaffrey and Horsburgh, 1986). Functional response has been classified into three basic categories suggested by Holling (1959). Most of the predators and parasitoids exhibit type II functional response but type III has been observed in some species too (Hassell *et al*. 1977; Hogvar and Hosvang, 1991; Fathipour *et. al*. 2001; Rakhshani *et al*. 2004; Askarianzade *et al*. 2009; Faal-Mohammad-Ali *et al*. 2010; Mostaghimi *et al*. 2010; Asadi *et al*., 2012; Tazerouni *et al*., 2012). Sarmento *et al*. (2007) found out that when the females of *Eriopis connexa* Germar (Coleoptera: Coccinellidae) preyed upon aphids, presented a type III functional response. This behavior changed to a type II when mites were offered as their prey. *Diaeretiella rapae* exhibited type III when *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) was used as its host (Jokar *et al*. 2012). Pandey *et al*. (1984) reported a sigmoid relationship between the number of eggs laid and the host density. Whereas, there are some reports of functional response type II for *D. rapae* in different host species and under variable conditions (De-Jiu *et al.,* 1991; Desneux *et al*., 2005; Fathipour *et al*. 2006; Tazerouni *et al*., 2011, 2012).

There are many factors such as pesticides that affect the functional response of a natural enemy (Oaten and Murdoch, 1975). Pesticides can alter the behavior of parasitoids through their neurotoxic activity, even in low doses. They may also reduce the parasitoid's behaviors such as searching capacity, response to host kairomones and pheromonal communication. Moreover, changes in foraging patterns and impairment of odor learning have been reported (Desneux *et al*., 2003).

There are some studies that confirm these effects (De-Jiu *et al.,* 1991; Desneux *et a*l., 2003; RafieeDastjerdi *et al*., 2009; Faal-Mohammad-Ali *et al*. 2010; Amini Jam *et al.* 2012; Sohrabi *et al.* 2012).

There is no previous study on sublethal effects of insecticides on *D. rapae* parasitizing *L. erysimi* on canola. The present study was designed to investigate the potential effects of pirimicarb and thiamethoxam on parasitoid *D. rapae* in order to find the proper insecticide in management programs of *L.erysimi*.

Materials and Methods

Host and parasitoid cultures

The mustard aphid *L. erysimi* and its parasitoid *D. rapae* were obtained from canola fields in February 2013 in Khuzestan province, southwestern Iran. They were maintained on canola seedlings *Brassica napus* L., cultivar 401 in muslin – walled cages (110 × 80 × 80 cm). The rearing conditions were at 25 ± 2 °C, 50-60% R. H and a photoperiod of 16: 8 h (L: D).

Insecticides

Insecticides tested were pirimicarb (Pirimor® 50 W.P, Moshkfam Fars, Iran) and thiamethoxam (Actara® 25 W. G, Syngenta, Switzerland).

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Bioassay

Residual bioassay was adopted in this study. The test concentrations were determined by preliminary experiments to find the lower and upper doses that cause 5-90% mortality. For each insecticide a water control and five concentrations with logarithmic intervals were prepared by diluting the stock solution in distilled water. Each concentration had five replications and all tests were repeated three times.

To obtain a homogeneous residual layer, 150 µl of each concentration and distilled water as control was applied on the inner side of glass vials (diameter: 4.5 cm; length: 7.5 cm).Vials were regularly rotated and left for 2 h to complete evaporation. Fifteen newly emerged female parasitoids (< 24 h old) were placed in each vial provided with honey solution (30% v/v). Pesticide exposure was carried out at 23 ± 2 °C, 70 ± 5 % RH and 16:8 h (L: D). After 24 h of exposure, the number of dead parasitoids was counted.

Data were analyzed by Polo-Plus software (LeOra Software, Version 2, 2013) to estimate LC_{50} values and their 95% confidence limits (C. L). The LC_{50} values of pesticides were compared by relative toxicity ratio test (Robertson *et al*., 2007; Heong *et al*., 2011).

Functional response

In order to expose the parasitoids to LC_{25} of insecticides, the same method mentioned in bioassay section was applied. LC_{25} was chosen as the sublethal dose because it has below 30% mortality threshold recommended in use of insecticides in IPM (Barrett *et al*., 1994; Bayram *et al*., 2010). The concentrations used for thiamethoxam and pirimicarb were 0.059 and 79.89 ppm, respectively. Seven parasitoids treated with LC_{25} of pesticides and distilled water as control were introduced to transparent cylindrical containers (diameter: 7.5 cm; length: 18 cm) individually. These containers enclosed canola seedlings in 4-5 leaf-stage infested by different densities (2, 4, 6, 8, 16, 32, and 64) of third instar nymphs of *L. erysimi*. This is the preferred nymphal instar of this species for *D. rapae* (Sing and Sinhat, 1982). Each density had 10 replicates. The containers were kept in an incubator under controlled conditions as in previous experiments. After 24 h the parasitoids were removed from experimental units and the aphids remained for about 10 days in order to count the mummies.

Functional response analysis

It is difficult to determine the type of functional response before fitting data to Holling and Rogers models (Holling, 1959; Rogers, 1972) so a logistic regression proportion of parasitized host versus the initial number of hosts offered, (N_a/N_0) is usually recommended (Juliano, 2001). Equation 1)

$$\frac{N_a}{N_0} = \frac{\exp\left(P_0 + P_0 N_0 + P_0 N_0^2 + P_0 N_0^3\right)}{1 + \exp\left(P_0 + P_0 N_0 + P_2 N_0^2 + P_3 N_0^3\right)}$$

Where N_a is the number of parasitized hosts and N_0 is initial host density. P_0, P_1,P_2 and $P_3$are the intercept, linear, quadratic and cubic, respectively. Maximum likelihood estimates of parameter P_0 to P_3 were obtained by applying the CATMOD procedure in SAS software (Juliano, 2001, SAS Institute, Inc. 2003). Type of functional response was determined by examining the sign of $P_1$and P_2. A negative and positive linear parameter (P_1) would indicate a type II and III, respectively (Juliano, 2001). As the host densities were depleted during the experiment, data fitted to Rogers' type II random parasitism equation (Rogers, 1972) (Proc NLIN; SAS Institute Inc. 2003).
Equation 2)

$$N_a = N_0\{1 - \exp[-a(T - ThN_0)]\}$$

Where *T* is the total time (24h), *a* is the attack rate (h^{-1}), and T_h is handling time in hours. Attack rate is the proportion of parasitized hosts versus total number of hosts existing in an experimental arena in a specific time unit. Handling time for a parasitoid includes time for host finding, oviposition, preening, resting and time for water or sap ingestion (Holling, 1959; Allahyari *et al.,* 2004). This parameter evaluates the ability of

a biological agent to allot its time to attack the host (Rogers, 1972). Pairwise comparisons of functional response's parameters for all possible pairs of treatments were performed by using the indicator variable method (Juliano, 2001) as follows:
Equation 3)

$$N_a = N_0\{1-\exp[-(a+D_{a(j)})(T-(Th+D_{Th(j)})))N_a]\}$$

Where j is an indicator variable which takes value 0 for the first treatment and 1 is for the second treatment. In order to test the significant differences in a and T_h between two treatments, the null hypothesis assumed that $D_a = 0$ and $D_{Th} = 0$, respectively. The parameters D_a and D_{Th} indicate the difference between a and T_h parameters in different treatments, respectively (Juliano, 2001; Allahyari *et al.,* 2004).

Results

Bioassay

The lethal dose values and upper and lower confidence limits of thiamethoxam and pirimicarb are presented in table 1. Hypothesis tests of parallelism (equal slopes) and equality (the sameness of slopes and intercepts) of the regression lines are rejected ($p < 0.05$) (Table 2).

In fifty percent lethality concentrations (LC_{50}), the relative toxicity ratio of thiamethoxam and pirimicarb was about 0.003 and their corresponding 95% confidence limits were 0.002-0.004, which did not include the value 1.0. Relative toxicity was considered significant ($P < 0.05$) when their 95% confidence intervals (C. I) did not include the value 1.0 (Robertson *et al*., 2007; Heong *et al*., 2011). In other words, the toxicity of thiamethoxam to *D. rapae* is significantly greater than pirimicarb.

Functional response

The functional response of *D. rapae* is shown in figure 1. In all experiments, the number of host parasitized increased with increasing the host densities. Fitting the polynomial logistic regression model (equation 1) to data set indicated that in both pesticides treatments and control, the linear parameter (P_1) value is negative. The P_1 values obtained for control, pirimicarb and thiamethoxam were -0.07 ±. 062 ($\chi^2 = 1.39$, $P_{value} = 0.24$), -0.07 ± 0.05 ($\chi^2 = 1.55$, $P_{value} = 0.21$), -0.10 ± 0.06 ($\chi^2 = 3.13$, $P_{value} = 0.08$).

Therefore, in order to compare the search rates (a) and handling time (T_h) of *D. rapae* on *L. erysimi*, the Rogers type II random parasitism model were fitted for each treatment data set. Estimated instantaneous attack rate (a) of *D. rapae* in control, pirimicarb and thiamethoxam were 0.057 ± 0.01 (0.040–0.080), 0.058 ± 0.013(0.03 – 0.08) and 0.04 ± 0.01 (0.02-0.06) h^{-1}, respectively. This parameter value was highest in control, pirimicarb and thiamethoxam, respectively. Comparisons of attack rate for all yielded no significant pairwise differences in attack rate (a) (Table 3).

The attack rate of control and pesticide treatments assumed to be a and $a \pm D_a$, respectively. If the confidence limits include value 0, there are no significant differences between these two treatments (Juliano, 2001).

Table 1 Comparative toxicity of thiamethoxam and pirimicarb tested on *D. rapae.*

Insecticide	n	Slope ± SE	$\chi2$ (df)	LC_{25} (ppm) (95% CL)[1]	LC_{50} (ppm) (95% CL)[1]	LC_{90} (ppm) (95% CL)[1]
Pirimicarb	1069	3.92 ± 0.23	0.46 (3)	79.89	118.71	251.9
				(73.71-85.69)	(112.12-125.58)	(229.94-281.26)
Thiamethoxam	1137	0.86 ± 0.05	11.35 (3)	0.06	0.39	11.23
				(0.02-0.14)	(0.15-0.89)	(3.52-88.9)

[1] CL: Confidence limits

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Diaeretiella rapae handling time (T_h) for control, pirimicarb and thiamethoxam, were 1.09 ± 0.098 (0.09–1.29), 1.86 ± 0.02 (1.53–2.19) and 2.81 ± 0.29 (2.22–3.40) h, respectively. Pairwise experiments demonstrated that parameter T_h significantly differed in all treatments (Table 3). The longest handling time related to thiamethoxam and pirimicarb, respectively.

The maximum number of attacks is limited by an upper asymptote value defined by the ratio of T/T_h (Hassell, 1978). So, the maximum number of parasitized aphids in control, pirimicarb and thiamethoxam were 21.87, 12.9 and 8.53 aphids during 24h, respectively.

The coefficient of determination (R^2 = 1-residual sum of squares / corrected total sum of square) indicated equal variation in parasitism rate of parasitoid (Allahyari *et al*., 2004; Farrokhi *et al.,* 2010). The R^2 values for control, pirimicarb and thiamethoxam were 0.92, 0.87 and 0.79, respectively.

Table 2 Hypothesis test of equality and parallelism of regression lines.

Hypothesis	$\chi 2$	df	P-value
Equality	708	2	0.000
Parallelism	234	1	0.000

Discussion

It is documented that the pesticides applied to control the pests cause negative effects on reproduction and behavioral activities of beneficial arthropods (Claver *et al.,* 2003; Poletti et *al.,* 2007; Gholamzadeh Chitgar*et al.,* 2013; Mahdavi *et al.,* 2013). Among the behavioral activities, functional response is widely used to evaluate the host-parasitoid interactions. Studies illustrated that sublethal doses of chemicals would change the type of functional response (Perera, 1982; De-Jiu *et al.,* 1991; Claver *et al.,* 2003). According to De-Jiu, *et al*. (1991) the sublethal doses of pirimicarb, cypermethrin and dimethoate on parasitoid *D. rapae* changed its functional response from type II (in control treatment) to type III. It was equally observed in *Acanthaspis pedestris* (Stal) (Reduviidae), which altered its functional response from type II to IV (dome–shape curve) in exposure to cypermethrin (Claver *et al.,* 2003).

The current study indicates that when the parasitoid *D. rapae*is is exposed to pirimicarb and thiamethoxam its functional response (type II) is not changed compared to the control treatment. These results agree with other published data in other parasitoid species (Saber *et al*., 2002; Thairi *et al*., 2007; Rafiee Dastjerdi *et al*., 2009; Amini Jam *et al*., 2012; Sohrabi *et al*., 2012; Mahdavi *et al.,* 2013).

According to the overlap in fiducial limits, thiamethoxam and pirimicarb did not affect the attack rate significantly. However, the handling time became longer in thiamethoxam and pirimicarb, respectively. Neural disorder in a parasitoid may involve prolongation of host finding, oviposition or preening. Hence, these results could be due to the mode of action of these insecticides which affects the neural system of parasitoids. Negative impacts of neonicotinoid insecticides on natural enemies and other non- target arthropods are well documented (Poletti *et al.,* 2007; Al Antary *et al*., 2010; Amini Jam *et al*., 2012; Sanchez-Bayo *et al*., 2013). These problems led to the banning of their application on sunflower, canola and corn crops (Sanchez-Bayo *et al*., 2013). For example the acute toxicity of thiamethoxam and imidacloprid to *Trichogramma chilonis* Ishii, is about 2000 times higher than that of other insecticides used in rice crops in India (Sanchez-Bayo *et al*., 2013). Whereas, pirimicarb is more toxic to cabbage aphid *Brevicoryne brassicae* L. than its parasitoid *D. rapae* in cabbage (Al Antary *et al*., 2010). Moreover, its lower toxicity on non-target arthropods such as Collembola in cereal crops has been reported (Sanchez-Bayo *et al*., 2013).

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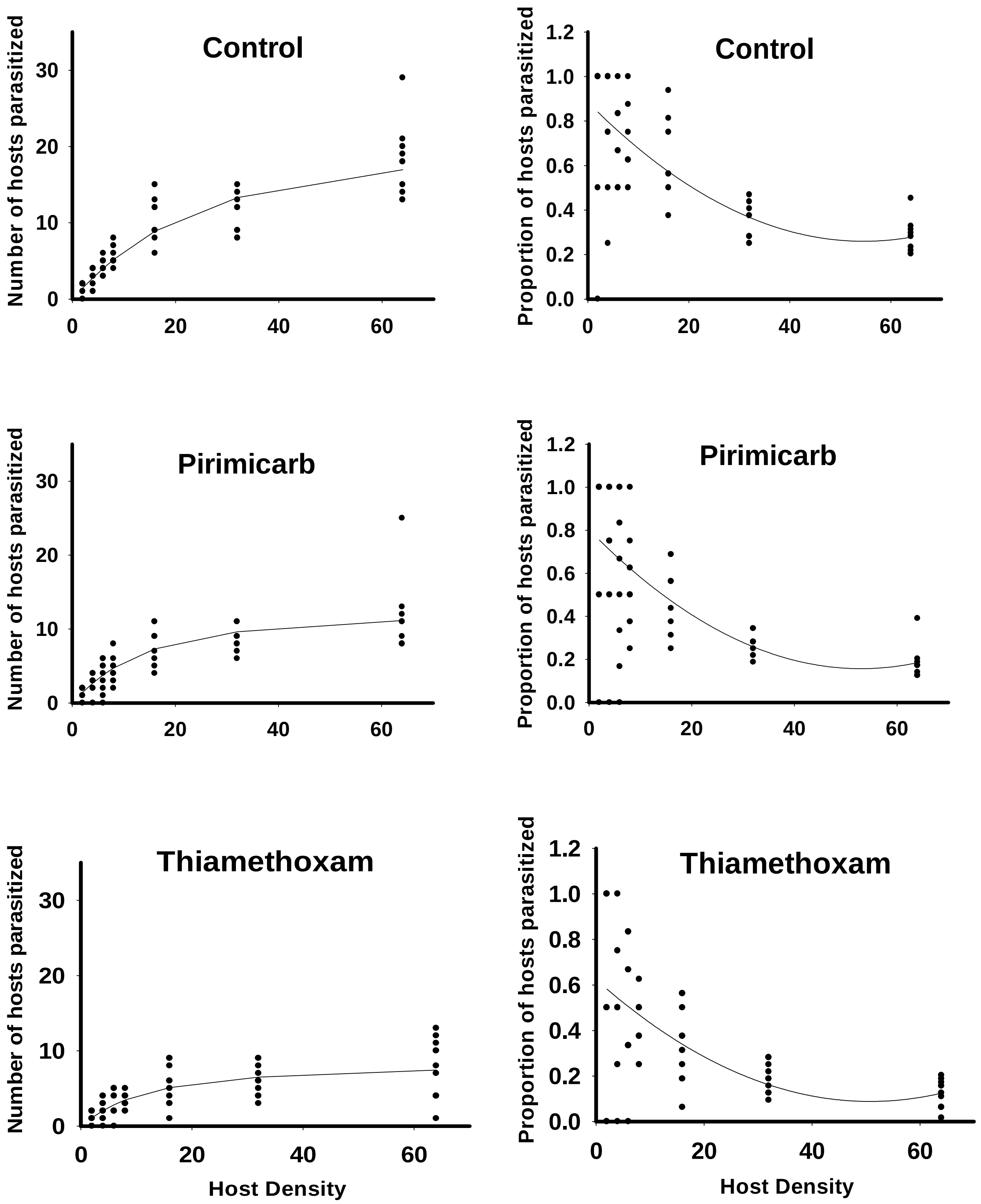

Figure 1 Functional responses of *D. rapae* in different densities of *L. erysimi*. Right: number of hosts parasitized (Symbols are observed data and lines are predicted by model (equation 2). Left: proportion of hosts parasitized.

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Table 3 Parameters estimated by an equation with indicator variable (3) for *D. rapae*.

Parameter	Estimate	Asymptotic (SE)	95% CI[1] (lower)	95% CI[1] (Upper)
Control and Pirimicarb				
Attack rate (a)	0.057	0.01	0.04	0.08
Handling time (T_h)	1.097	0.09	0.91	1.29
D_a[2]	0.002	0.02	-0.03	0.03
D_{Th}[3]	0.760	0.19	0.38	1.15
Control and Thiamethaxam				
Attack rate (α)	0.060	0.01	0.04	0.07
Handling time (T_h)	1.097	0.09	0.92	1.28
D_a	-0.020	0.01	-0.04	0.01
D_{Th}	1.710	0.34	1.04	2.39
Thiamethaxam and Pirimicarb				
Attack rate (α)	0.040	0.01	0.02	0.06
Handling time (T_h)	2.820	0.32	2.19	3.44
D_a	0.019	0.02	-0.01	0.05
D_{Th}	-0.950	0.35	-1.65	-0.26

[1] CI: Confidence interval.
[2] D_a: indicator variable estimates the differences between the treatments in the value of the parameter a.
[3] D_{Th}: indicator variable estimates the differences between the treatments in the value of the parameter T_h.

By evaluating the maximum rate of parasitized aphids it is demonstrated that the efficiency of parasitism in control treatment is 1.7 and 2.6 times more than its value in pirimicarb and thiamethoxam, respectively.

In order to protect the reach fauna of beneficial arthropods (such as pollinators and parasitoids) in canola, the crop management is very crucial. Hence, pirimicarb is preferred for chemical control of *L. erysimi* in canola. Routinely, functional response experiments, like present study, are carried out in controlled conditions. However, it is obvious that in the field conditions, natural enemies encounter unstable and highly variable conditions. On the other hand, it is difficult to predict the natural enemies' reactions to these changes. Therefore, it is suggested to perform such studies under semi field and field conditions apart of laboratory experiments.

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اثر دزهای زیرکشنده تیامتوکسام و پریمیکارب روی واکنش تابعی زنبور *Diaeretiella rapae* (Hymenoptera: Braconidae) پارازیتوئید شته خردل *Lipaphis erysimi* (Hemiptera: Aphididae)

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چکیده: سمیت حشره‌کش‌های تیامتوکسام و پیریمیکارب روی زنبور *Diaeretiella rapae* (Mc'Intosh) پارازیتوئید شته خردل *Lipaphis erysimi* (Kaltenbach) در شرایط آزمایشگاهی ۲ ± ۲۳ درجه سیلسیوس، رطوبت نسبی ۵ ± ۷۰٪ و دوره نوری ۱۶:۸ ساعت (تاریکی/روشنایی) بررسی شد. همچنین به-منظور بررسی اثرات منفی حشره‌کش‌های مذکور بر کارایی پارازیتوئید *D. rapae*، واکنش تابعی زنبورهای تیمار شده مورد ارزیابی قرار گرفت. پارازیتوئیدهای تازه بالغ شده در معرض LC_{25} حشره‌کش‌ها و آب مقطر به‌عنوان شاهد، قرار گرفتند. گیاهچه‌های کلزای آلوده به تراکم‌های مختلف پوره سن سوم شته خردل (۴،۲، ۶، ۸، ۱۶، ۳۲ و۶۴) در ۱۰ تکرار دراختیار زنبورهای تیمارشده قرار گرفت. نوع واکنش تابعی با استفاده از معادله رگرسیون لجستیک تعیین گردید و پارامترهای نرخ حمله *a* و زمان دستیابی T_h با استفاده از مدل رگرسیون خطی با استفاده از نرم‌افزار SAS محاسبه شد. پارازیتوئید *D. rapae* در تمام تیمارها، واکنش تابعی نوع دوم را نشان داد. نرخ حمله در شاهد، پیریمیکارب و تیامتوکسام به‌ترتیب ۰/۰۱ ± ۰/۰۵۷و ۰/۰۱۳ ± ۰/۰۵۹ و ۰/۰۱ ± ۰/۰۴۰ بر ساعت و زمان دستیابی ۰/۱ ± ۱/۰۹۷ و ۰/۰۲ ± ۱/۸۶ و ۰/۲۹۶ ± ۲/۸۱ ساعت بود. حداکثر نرخ پارازیتیسم (T/T_h) به‌ترتیب ۲۱/۸۷ و ۱۲/۹ و ۸/۵۳ محاسبه گردید. براساس نتایج این آزمایش پیشنهاد می‌شود که پیریمیکارب با داشتن اثرات منفی کمتر، گزینه مناسب‌تری برای کنترل شته خردل می‌باشد.

واژگان کلیدی: *Diaeretiella rapae*, *Lipaphis erysimi*، واکنش تابعی، Pirimicarb، Thiamethoxam

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