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Research Article

Effects of diazinon and fipronil on functional response of *Trichogramma brassicae* Bezdenko (Hym.; Trichogrammatidae) in the laboratory conditions

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Abstract: *Trichogramma* is an important genus of egg parasitoids that is frequently used as biological control agents against lepidopteran pests. The most widespread species of *Trichogramma* in Iran is *Trichogramma brassicae* Bezdenko that is widely used against rice stem borrer, *Chilo suppressalis* (Walker). In this study, the sublethal effects of LC_{30} concentration of diazinon and fipronil were studied on the functional response of *T. brassicae* to different densities (2, 4, 8, 16, 32 and 64) of *Sitotroga cerealella* (Olivier) eggs. The experiment was carried out in an insectarium at 25 ± 1 ºC, 70 ± 10% RH, and a photoperiod of 16: 8 (L: D) h. Young adult females of the parasitoid were exposed to LC_{30} of either insecticides for an appropriate time of exposure. Then, fresh host egges were offered to survived female wasps for parasitim for 24 h. The type of functional response was determined using logistic regression and the parameters including searching efficiency (a) and handling time (T_h) were estimated by non-linear regression. The results revealed a type II functional response in the control and fipronil, and type III for diazinon. In this study, application of insecticides caused a decrease in the attack rate and an increase in the handling time of exposed wasps compared with the control. The longest handling time (3.76 ± 0.4 h) and the lowest attack rate (0.001 ± 0.0004) were observed in diazinon. The results suggested that the adverse effect of this insecticide on searching ability of *T. brassicae* should be considered in integrated pest management programs (IPM).

Keywords: behavioral effect, biological control, egg parasitoid, insecticide

Introduction

Rice stem borrer, *Chilo suppressalis* (Walker) is the most important pest of rice in northern region of Iran. This pest was introduced in 1973 and has been widely distributed in all rice fields of the country and has caused economic damage durning four past decades (Khanjani, 2006). The pest attacks the rice plant in different developmental stages causing symptoms known as dead heart and white head (Rubia-Sanchez *et al.,* 1997). Various tactics, including varietal resistance, Bt rice, cultural practices, chemical and biological control, are used to control rice stem borer. Chemical control is still preferred by farmers and insecticides such as diazinon

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(Ghassempour *et al.,* 2002) and fipronil (Talebi Jahromi, 2007) have been extensively used in rice fields. The extensive and repeated use of insecticides could cause serious problem such as possible toxicity in humans and animals, side effects of pesticides on nontarget organisms, secondary pest outbreaks, development of insecticide resistance and environmental pollution (Talebi Jahromi *et al.,* 2011). Biological control may reduce pesticide applications (Landis *et al.,* 2000, Frank, 2010). Among the biological control agents, the *Trichogramma* spp. occurring worldwide, play an important role as natural enemies of lepidopterous pests on a wide range of agricultural crops (Abdelgader and Hassan, 2012). The short generation time of *Trichogramma* spp, and the fact that they can be reared on factitious hosts, allows these wasps to be produced quickly and affordably relative to the other parasitoids (Li, 1994; Smith, 1996). The most widespread species in Iran is *Trichogramma brassicae* Bezdenko (Azema and Mirabzadeh, 2005). This natural enemy may be affected by insecticide sprays in rice fields via direct contact with residues, or indirectly through contaminated food. Integrating the application of biocontrol agents and insecticides for Integrated Pest Management (IPM) in rice ecosystem requires knowledge about impact and selectivity of the insecticides on natural enemies (Dent, 1995; Croft, 1990). Several biological characteristics, including searching ability, fecundity, longevity and sex ratio have been used to assess potential efficacy of a parasitoid. In the case of *Trichogramma*, the number of host eggs successfully parasitized by the adult female parasitoid (fecundity) after release in the field is the key attribute for selecting species or strains. Another important aspect when evaluating the efficiency of a natural enemy is the attack rate across a range of densities of the host, i.e., its functional response (Berryman, 1999). The functional response is an essential element of dynamics of host-parasitoid association and is an important determinant of the stability of the system (Oaten and Murdoch, 1975). Holling (1959a, b, 1966), developed mathematical models to describe natural enemy responses to changing prey or host density, initially described as “functional response”. In type I functional response, number of killed host/prey rises linearly to a plateau; in type II, a curvilinear rise to a plateau is present and in type III host mortality increases by a sigmoid trend (Hassell, 2000; Mills and Lacan, 2004). Functional response experiments show the potential ability of a parasitoid/predator to suppress the different densities of prey/host (Moezipour *et al.,* 2008). The functional response of *Trichogramma* species has generally been found to be either type I or type II (Smith, 1996). However, a type III response has also been reported (Wang and Ferro, 1998).

In this study, we evaluated the sublethal effects of two insecticides, diazinon and fipronil on the functional response of *T. barssicae.* Such information can be used to predict the potential of these insecticides in combination with *T. brassicae* for controlling rice pests.

Materials and Methods

Parasitoid cultures

Adult *T. brassicae* were obtained from an insectarium belonging to the Rice Research Institute of Iran, Guilan (North of Iran). The parasitoid species had been identified by Dr. Ebrahimi at Iranian Research Institute of Plant Protection. *Trichogramma* wasps were reared on *Sitotroga cerealella* (Olivier) eggs for eight generations in an insectarium at 25 ± 1 ºC, 70 ± 10% RH, and a photoperiod of 16:8 (L: D) h. Honey was presented as food for the adult parasitoids on a stripe of paper (Rafiee-Dastjerdi *et al.,* 2009).

Insecticides

The insecticides used in the experiments were diazinon (Diazinon Aria® 60% EC, Ariashimi, Iran) and fipronil (Rigent® 20% G, Partonar Shimi, Iran).

Functional response experiment

Mated young adult females (< 24 h old) of *T. brassicae* were exposed to LC_{30} of diazinon or fipronil insecticides (0.01 and 1.07 ppm) by using exposure cages (Saber *et al.,* 2005). The glass surfaces of the cages were sprayed with aqueous solutions of the LC_{30} concentration of the insecticides. Tween 80 (Merck Darmstadt, Germany) was used as the wetting agent in all dilutions (Rosenheim and Hoy 1988). The controls were sprayed with distilled water plus Tween 80. Before completely assembling the cages, 200 ± 20 mated young adults (< 24 h old) were introduced in each cage. Twenty-four hours after treatment with the LC_{30} concentration of each insecticide, 10 survived female adults were randomly selected and transferred individually to glass tubes bearing 2, 4, 8, 16, 32 or 64 *S.cerealella* eggs, and the wasps were provided with honey as food. After 24h, the wasps were removed and the glass tubes containing parasitized eggs were kept in a growth chamber for 8 days in oreder to assess parasitism. Experiments were performed in 10 replications for each treatment as well as control.

Statistical analysis

Analysis of functional response data consisted of two distinct steps (Messina and Hanks 1998; De Clercq *et al*., 2000; Juliano, 2001; Mohaghegh *et al.,* 2001; Allahyari *et al.,* 2004). In the first step, the curve shape or type of functional response was determined, typically by determining if the data fit a type II or III functional response. The parameters to be estimated were P0, P1, P2 and P3. These parameters were estimated using the Maximum Likelihood Analysis (CATMOD) procedure in SAS software (Juliano, 2001). In the second step, a nonlinear least square regression was used [Nonlinear (NLIN) following the procedure with Multivariate Secant or False Position (DUD) method in SAS (2002)] to estimate the functional response parameters of the Holling's disc equation (Williams and Juliano, 1985). Then, the obtained parameters were compared [T_h and either a (for type II)]. The coefficient of determination was calculated as $r^2 = 1 -$ residual sum of squares/corrected total sum of squares (Allahyari *et al*., 2004; Farrokhi *et al*., 2010).

Results

The LC_{30} values of each insecticide on *T. brassicae* are presented in Table 1. Functional responses of parasitoid in exposure to diazinon and fipronil were shown in figures 1 and 2.

Parameter estimates for logistic regressions of all treatments are presented in Table 2. In the logistic regressions, if linear parameter P1 was negative, it would show a type II functional response, whereas a positive linear parameter might indicate a type III functional response. Results of logistic regression (Table 2) indicated that functional response of *T. brassicae* in diazionon and fipronil-treated populations were type III and II, respectively.

Table 1 Estimation of LC_{30}, confidence limit and dose-response data for diazinon and fipronil on *Trichogramma brassicae.*

Insecticides	n	LC_{30} (ppm) or [µg a.i/ml] (95% FL)	Slope ± SE	χ^2
Diazinon	200	0.01 [0.006] (0.007 – 0.03)	0.53 ± 0.07	37.07
Fipronil	200	1.07 [0.00214] (0.91 – 1.22)	1.58 ± 0.10	72.93

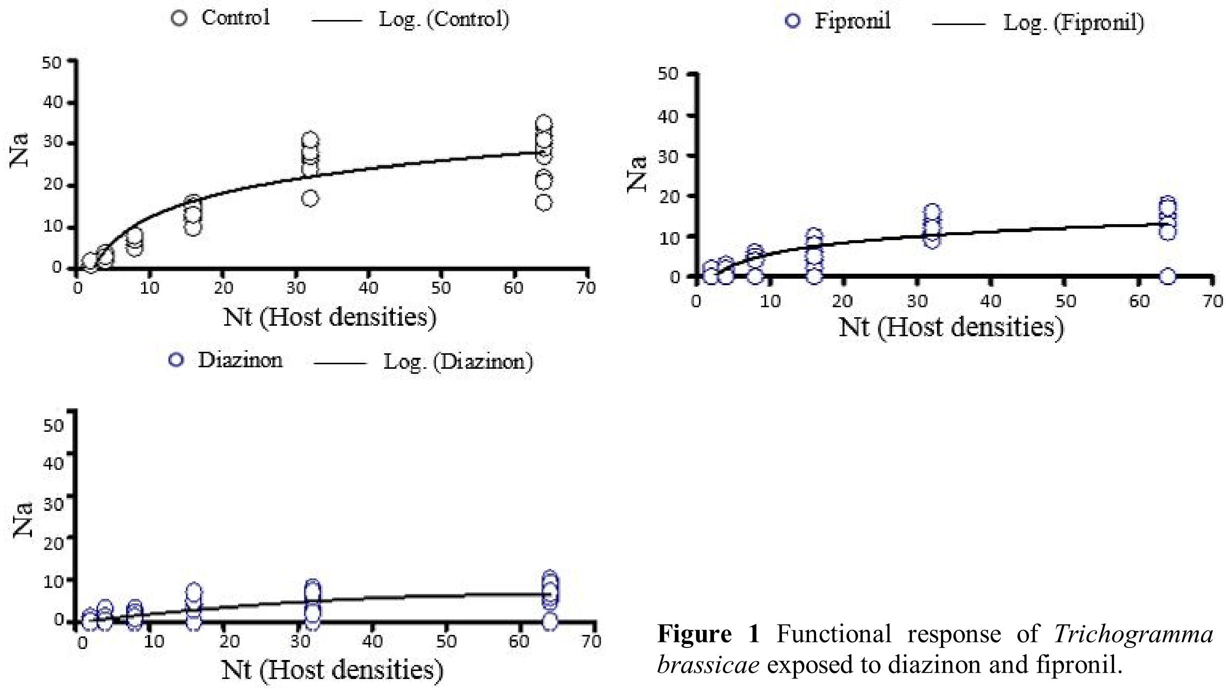

Figure 1 Functional response of *Trichogramma brassicae* exposed to diazinon and fipronil.

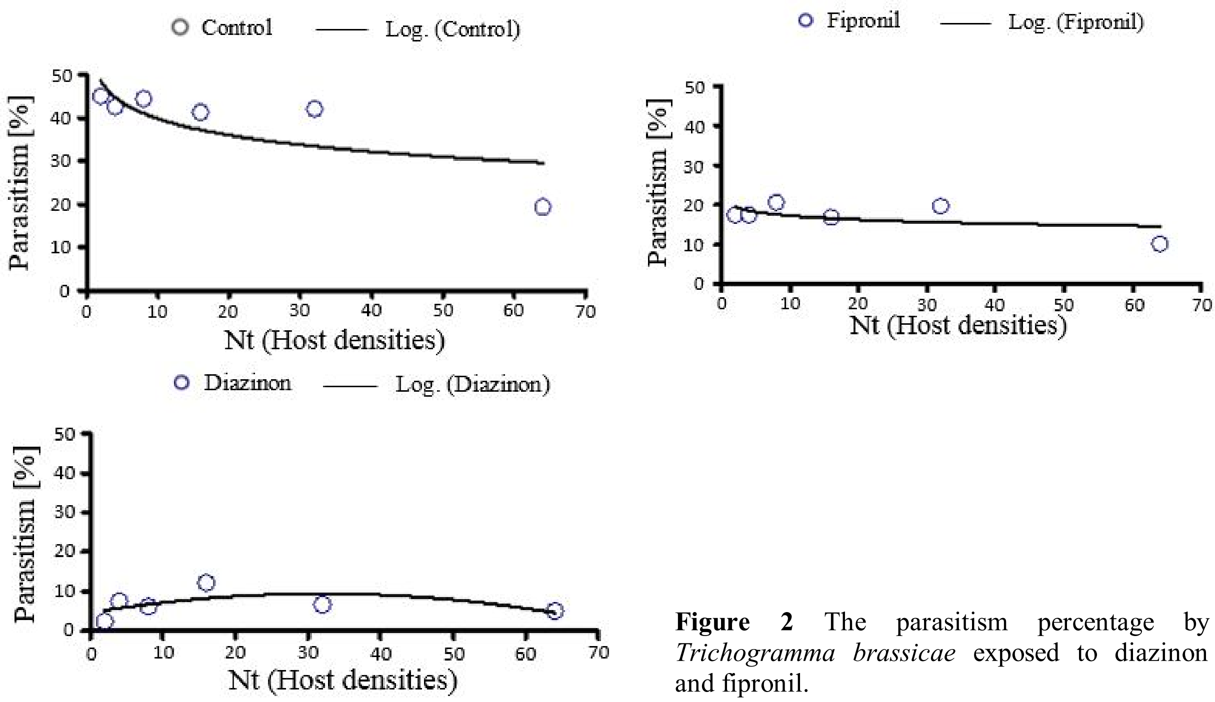

Figure 2 The parasitism percentage by *Trichogramma brassicae* exposed to diazinon and fipronil.

The handling times and coefficient of attack rates are depicted in Table 3. This study showed that control and diazinon had the lowest (0.7852 ± 0.0306 h) and highst (3.7586 ± 0.04036 h) handling time, respectively. The highest and lowest value of attack rate was observed in control (0.00508 ± 0.0006 per hour) and diazinon (0.00110 ± 0.000389 per hour), respectively.

Table 2 Results of the logistic regression analysis of the proportion of *Sitotroga cerealella* eggs parasitised by *Trichogramma brassicae* in the initial density.

Treatment	Parameters	Estimate ± SE	χ^2	p – value
Control	P0 (constant)	2.3298 ± 0.5705	16.68	< 0.0001
	P1 (linear)	- 0.0915 ± 0.0831	1.21	0.2707
	P2 (quadratic)	0.0036 ± 0.0031	1.37	0.2425
	P3 (cubic)	- 0.00004 ± 0.00003	2.11	0.1465
Diazinon	P0 (constant)	- 3.0197 ± 0.6024	25.13	< 0.0001
	P1 (linear)	0.2376 ± 0.0837	8.05	0.0046
	P2 (quadratic)	- 0.00907 ± 0.00306	8.77	0.0031
	P3 (cubic)	0.000087 ± 0.00003	8.47	0.0036
Fipronil	P0 (constant)	-0.3712 ± 0.3836	0.94	0.3331
	P1 (linear)	- 0.0388 ± 0.0586	0.44	0.5077
	P2 (quadratic)	- 0.00193 ± 0.00222	0.76	0.3840
	P3 (cubic)	- 0.00002 ± 0.000022	1.25	0.2629

Table 3 Functional response parameters estimated for *Trichogramma brassicae* exposed to the insecticides and control.

Treatment	Functional response type	a ± SE (h^{-1}) (lower-upper)	T_h ± SE (h) (lower-upper)	T/T_h	r^2 at $p < 0.001$
Control		0.00508 ± 0.00060	0.7852 ± 0.3060	30.37	0.85
	II	(0.00379 – 0.00637	(0.7239 – 0.8464		
Diazinon		0.00110 ± 0.00039	3.7586 ± 0.4036	6.38	0.04
	III	(0.00032 – 0.00188	(2.9508 – 4.5665		
Fipronil		0.00204 ± 0.00046	1.6453 ± 0.1183	14.63	0.57
	II	(0.00113 – 0.00295)	(1.4084 – 1.8822)		

Discussion

The negative and positive values for the linear parameters obtained in this study confirmed type II and III functional response for fipronil and diazinon-treated wasps, respectively. The type II and III functional responses are common among arthropod predators (Hassell *et al.,* 1977). These results are consistent with the results by Moezipour *et al.* (2008) who found that functional response of *T. brassicae* on its factitious host, eggs of *Sitotroga cerealella* at 25 °C was type II and at 20 °C and 30 °C was type III. Mahdavi-ParchinSofla (2011) also examined the sublethal effects of abamectin, carbaryl, chlorpayrifos and spinosad on the functional response of ectoparasitoid *H. hebetor*, and reported a type III functional response for all treatments and the control. GholamzadehChitgar *et al.,* (2013) tested the sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug*, Andrallus spinidens* Fabricius (Hem.: Pentatomidae), and reported that the functional response of the predator treated with pesticides was type II in control and all insecticide treatments.

Functional response manifests two important parameters including a and T_h used to evaluate the effectiveness of predators and parasitoids (Hassell and Waage, 1984). The rate of these parameters and the type of functional response in predators and parasitoids are influenced by different factors. One of them is the sublethal concentrations of insecticides (Rafiee-Dastjerdi *et al.,* 2009; Ambrose *et al.,* 2010). In this study, diazinon and fipronil showed sublethal effects on functional response parameters of *T. brassicae* including searching efficiency and handling time. Our findings demonstrated that the wasps exposed to insecticides had a higher handling time (Table 3), in which the highest T_h occurred in the diazinon treatment. Moreover effects of insecticides revealed that the value of this parameter decreased with insecticide application and the lowest value was observed in the parasitoid exposed to diazinon.

Pesticides used in this study have been widely used to control rice lepidopter pests in Iran. It is reported that organophosphates have low selectivity to natural enemies (Fernandes *et al.,* 2010). Therefor, The results obtained in this study may be useful for the evaluation of rice field insecticides on *T. brassicae* as biological control agent of lepidopteran pests. Also, functional response studies in laboratory could be useful in providing the first step for comparing the efficiency of different species/strains and also provide a valid means of comparing host finding abilities of candidate natural enemies (Overholt and Smith, 1990). Overall, the results showed that fipronil had less adverse effects on behavior of the parasitoid compared to diazinon and would be applied in rice fields when Trichogramma wasps are used against rice stem borrer. In addition to laboratory studies, more attention should be devoted to semi field and field conditions to obtain more applicable results in the field.

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اثر دیازینون و فیپرونیل روی واکنش تابعی زنبور پارازیتوئید تخم *Trichogram brassicae* Bezdenko (Hym.; Trichogrammatidae) در شرایط آزمایشگاهی

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چکیده: تریکوگراما یکی از جنس‌های مهم پارازیتوئیدهای تخم است که به فراوانی به‌عنوان عامل کنترل بیولوژیک آفات بالپولکی استفاده می‌شود. شایع‌ترین گونه تریکوگراما در ایران، *Trichogram brassicae* Bezdenko است که به‌طور گسترده‌ای برای کنترل کرم ساقه‌خوار برنج *Chilo suppressalis* (Walker) در مزارع برنج شمال کشور استفاده می‌شود. در این مطالعه اثرات زیرکشندگی LC_{30} دیازینون و فیپرونیل روی واکنش تابعی *T. brassicae* به تراکم‌های مختلف (۲، ۴، ۸، ۱۶، ۳۲ و ۶۴) تخم‌های بید غلات *Sitotroga cerealella* (Olivier) مطالعه شد. آزمایشات در یک اتاق رشد با دمای ۱ ± ۲۵ درجه سلسیوس، رطوبت نسبی ۱۰ ± ۷۰ درصد و دوره نوری ۸: ۱۶ ساعت (روشنایی: تاریکی)، انجام گرفت. حشرات کامل جوان پارازیتوئید در معرض LC_{30} هر یک از حشره‌کش‌ها به‌مدت معین (بسته به زمان در معرض قراردهی محاسبه LC_{30}) قرار داده شدند. سپس تراکم‌های مختلف میزبان به‌مدت ۲۴ ساعت جهت پارازیتیسم در اختیار زنبوران ماده زنده مانده قرار داده شدند. نوع واکنش تابعی با استفاده از رگرسیون لجستیک و پارامترهای کارائی جستجوگری (a) و زمان دستیابی (T_h) به‌وسیله رگرسیون غیرخطی تعیین شد. نتایج نشان داد که واکنش تابعی در شاهد و فیپرونیل از نوع دوم و در تیمار دیازینون از نوع سوم بود. در این مطالعه، کاربرد حشره‌کش‌ها باعث کاهش در نرخ حمله و افزایش زمان دستیابی در مقایسه با شاهد شد. طولانی‌ترین زمان دستیابی (۰/۴ ± ۳/۷۶ ساعت) و کم‌ترین نرخ حمله (۰/۰۰۰۴ ± ۰/۰۰۱) در تیمار دیازینون مشاهده شد. براساس نتایج به‌دست آمده، بایستی اثرات منفی جانبی این حشره‌کش برروی جستجوگری زنبور تریکوگراما، در برنامه‌های مدیریت تلفیقی آفات مورد توجه قرار گیرد.

واژگان کلیدی: اثر رفتاری، پارازیتوئید تخم، حشره‌کش، کنترل بیولوژیکی