



ORIGINAL ARTICLE

# Sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae) in the laboratory conditions

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**Abstract** The sublethal effects of diazinon, fenitrothion and chlorpyrifos on the functional response of predatory bug, *Andrallus spinidens* Fabricius (Hem.: Pentatomidae), a potential biological control agent, were studied on 5th-instar nymphs. The experiment was conducted in varying densities (2, 4, 8, 16, 32 and 64) of last instars larvae of *Chilo suppressalis* Walker (Lepidoptera: Pyralidae) as prey at  $25 \pm 2$  °C,  $60\% \pm 10\%$  relative humidity (RH) and a photoperiod of 16:8 h (L: D). The results of logistic regressions revealed a type II functional response in the control and all insecticide treatments. Comparison of functional response curves revealed that tested insecticides markedly decreased the mean of preys consumed by *A. spinidens*. Among them, functional response curve of *A. spinidens* in chlorpyrifos treatment was significantly lower than the other treatments. In this study, application of insecticides caused a decrease in the attack rate and an increase in the handling time of exposed bugs compared with the control. The longest handling time ( $3.97 \pm 0.62$ ) and the lowest attack rate ( $0.023 \pm 0.007$ ) were observed in chlorpyrifos and fenitrothion treatments, respectively. The results suggested that the adverse effect of these insecticides on *A. spinidens* should be considered in integrated pest management programs (IPM).

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## 1. Introduction

Rice is an important crop and is cultivated mainly in the north of Iran at Mazandaran, Guilan and Golestan provinces. It is attacked by very destructive pests like *Chilo suppressalis* Walker (Pyralidae), *Naranga aenescens* Moore (Noctuidae) and *Mythimna unipunctata* Haworth (Noctuidae). Among them,

the rice striped stem borer, *C. suppressalis* is one of the most serious pests of rice. Khan et al. (1990) reported that the stem borers are major pests in all rice ecosystems. The pests attack the rice plant in different developmental stages causing symptoms like dead heart and white head (Rubia-Sanchez et al., 1997). The chemical control has been a prevalent tool for controlling these lepidopterous pests. Diazinon, fenitrothion and chlorpyrifos have been used extensively in rice fields (Ghassempour et al., 2002). More than 60% of chemical pesticides were used in Northern provinces of Iran against rice pests. In these regions, pesticides are applied 2–4 times during the rice cropping season (Noorhosseini, 2010). The extensive and repeated use of pesticides could cause serious problem such as possible toxicity in humans and animals. Further, side effects of pesticides on non-target organisms, secondary pest outbreaks, development of insecticide resistance and environmental pollution are also of concern (Talebi et al., 2011). For example, the residue of diazinon which is commonly used to control *C. suppressalis* was detected in the soil and surface-water of rice fields in the north of Iran. The studies have also shown that several useful soil microorganisms failed to grow on a medium containing diazinon (Ghassempour et al., 2002). In the rice ecosystem, natural enemies include predators and parasitoids, which are considered as important biological agents for controlling various insect pests. Conservation of natural enemies in the rice fields may suppress the pest populations, which in turn will reduce the rate of insecticide application (Jadhao, 2011).

*Andrallus spinidens* Fabricius is a non-specific predator on lepidopterous larvae in rice fields (Manley, 1982). Second to fifth instar nymphs and adults of *A. spinidens* have predatory activity on caterpillar pests of rice like *C. suppressalis*, *N. aenescens* and *M. unipunctata* (Nageswara Rao, 1965; Manley, 1982; Mohaghegh and Najafi, 2003; Behera and Prakash, 2004). This pentatomid bug has a critical role in the regulation of rice pest's population (Najafi-Navaee et al., 1998). There are three factors which should favor *A. spinidens* as a potentially useful biological control agent of rice pests: relatively short life cycle, aggressive feeding behavior and ability to feed continually for several hours (Manley, 1982). 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 (Croft, 1990; Dent, 1995).

The control of a pest by a predator depends strongly on the predator–prey interaction such as the predator's numerical and functional responses (Holling, 1959). Functional response tests show the potential of parasitoid/predator ability to suppress the different densities of prey/host. In fact, it describes the way a natural enemy responds to the changing densities of its prey and it is a commonly measured attribute of natural enemies. Holling (1959, 1966) proposed three types of functional responses. In type I, number of killed host/prey rises as linear to a plateau; type II, a curvilinear rise to a plateau which then levels off under the influence of handling time or satiation and in type III predator/parasitoid response by a sigmoid increase in prey/hosts attacked (Hassell, 2000; Mills and Laca, 2004). Many factors such as pesticides influence the functional response of a predator (Murdoch and Oaten, 1975). Several studies provided a strong evidence that

insecticides affect the functional response of natural enemies (e.g. Gu, 1991; Jebanesan, 1998; Wang and Shen, 2002; Claver et al., 2003; Deng et al., 2007; Ambrose et al., 2008, 2010; Raffiee-Dastjerdi et al., 2009; Rezac et al., 2010; Abedi et al., 2012). The functional response of *A. spinidens* to different densities of larvae of *N. aenescens* has been studied by Javadi and Sahragard (2005). However, there is no data about the investigation of insecticides on functional response of the predatory bug. In this study, we examined the sublethal effects of three insecticides, diazinon, fenitrothion and chlorpyrifos on the functional response of *A. spinidens*. Such information can be used to predict the potential of these pesticides in combination with *A. spinidens* in controlling rice pests.

## 2. Materials and methods

### 2.1. Insect rearing

The adults and nymphs of *A. spinidens* were collected from rice fields in Amol, Mazandaran province (north of Iran), in late September 2012. These insects were reared on last larval instar of *Galleria melonella* Linnaeus (Lep.: Pyralidae) in the laboratory conditions ( $25 \pm 2$  °C,  $60\% \pm 10\%$  RH and a photoperiod of 16:8 h L: D) h. The second generation of *A. spinidens* was used for experiments. Also, pupae of *C. suppressalis* were collected from the rice field in late May 2012 and kept in rearing chamber as above. After the emergence of adults and subsequent copulation and egg laying, the hatched larvae were reared on the rice seedling *Oryza sativa* L. (Taroum variety). The last larval instar of *C. suppressalis* was used as prey for functional response experiment of *A. spinidens*.

### 2.2. Pesticides

The pesticides used in this study were technical material of diazinon (Gyah Corporation, Iran, 99.8% purity), fenitrothion (Pesticides and Agriculture Research Center, Iran, 99.8% purity) and chlorpyrifos (ACO, USA, 99.9% purity).

### 2.3. Bioassay

Initially, preliminary bioassays were conducted to determine the effective concentrations caused between 10% and 90% mortality. The insecticides were bioassayed at serial concentrations in ranges of 1000–3500, 200–800 and 200–950 ppm a.i., for diazinon, fenitrothion and chlorpyrifos, respectively. These insecticides were diluted in acetone (Merck Company, Germany) and 1  $\mu$ l of each concentration was applied topically using a microapplicator on the thoracic dorsum of newly molted 5th-instar nymphs of *A. spinidens*. Control treatment received 1  $\mu$ l of acetone alone. Forty nymphs of *A. spinidens* were used for each concentration and the control. Mortality was assessed 24 h after treatment and the LC<sub>30</sub> value of each insecticide was estimated.

### 2.4. Functional response assay

In order to evaluate the searching efficiency of *A. spinidens*, the functional response of this predator to different densities of *C. suppressalis* was studied. Twenty-four hours after treatment

with the sublethal concentration, LC<sub>30</sub> of each insecticide, the surviving nymphs were kept separately without food for 12 h. Then, they were individually transferred to Petri dishes (60 mm in diameters) and were fed with different densities (2, 4, 8, 16, 32, and 64) of last instar larvae of *C. suppressalis*. After 24 h, the predators were removed and the number of consumed prey (Na) was evaluated. Each concentration was replicated five times.

### 2.5. Data analysis

The LC<sub>30</sub> values and 95% confidence intervals were calculated from probit regressions using the POLO-PC computer program (Leora Software, 1987). The type of the functional response was determined by logistic regression analysis (SAS/STAT, CATMOD procedure) of the proportion of prey killed ( $N_e$ ) in relation to initial prey density ( $N_0$ ) (Trexler and Travis, 1993). The data were fitted to the logistic regression which describes the relationship between  $N_a/N_0$  and  $N_0$  (Juliano, 1993):

$$\frac{N_e}{N_0} = \frac{\exp(P_0 + P_1N_0 + P_2N_0^2 + P_3)}{1 + \exp(P_0 + P_1N_0 + P_2N_0^2 + P_3)}$$

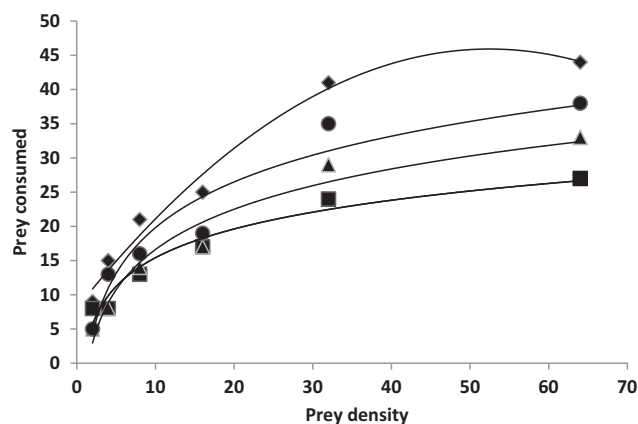
where  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the intercept of linear, quadratic and cubic coefficients, respectively, and estimated using the method of maximum likelihood. If the linear parameter  $P_1$  is negative, a type II functional response is evident, whereas a positive linear parameter indicates density-dependent predation and thus a type III functional response (Juliano, 1993).

After the determination of the shape of the curve, the handling times and attack coefficients of a Type II response were estimated using Holling's disk equation (Williams and Juliano, 1985). Statistical analysis of the functional response was performed using the SAS software (SAS Institute, 2002).

### 3. Results

The LC<sub>30</sub> values of each insecticide on *A. spinidensis* are presented in Table 1. The rank order of the toxicity, from the highest to the lowest, was fenitrothion > chlorpyrifos > diazinon.

The relationship between number of prey density and number of prey consumed for all treatments is illustrated in Fig. 1. In this figure, the response curve rises in a negatively accelerating manner to a plateau that showed type II of functional response. The proportion of preys consumed by a predator with a type II functional response decreases exponentially as the prey density increases. Type II is curvilinear and the saturation level is reached in a gradual manner. Comparison of functional response curves revealed that functional response curve of *A. spinidensis* in chlorpyrifos treatment was significantly lower



**Fig. 1** Type II functional response of *A. spinidensis* exposed to the LC<sub>30</sub> of different insecticides and control to densities of last larval instar of *C. suppressalis*. Chlorpyrifos (■), fenitrothion (▲), diazinon (●) and Control (◆).

than the other treatments. The mean number of prey consumed at the density of 64 larvae was significantly different among the treatments ( $F = 7.48$ ;  $df = 3$ ;  $P < 0.0024$ ).

Parameter estimates for logistic regressions of all treatments are presented in Table 2. In the logistic regressions, if linear parameter  $P_1$  was negative, it could show a type II functional response, whereas a positive linear parameter could indicate density-dependent predation and thus a type III functional response.

The values of searching efficiency ( $a'$ ) and handling time ( $T_h$ ), estimated by Rogers random attack equation are depicted in Table 3. The longest handling time ( $3.97 \pm 0.62$ ) and the lowest attack rate ( $0.023 \pm 0.007$ ) were observed in chlorpyrifos and fenitrothion treatments, respectively.

### 4. Discussion

The negative values for the linear parameters ( $P_1 < 0$ ) obtained in this study confirm type II functional response for all treatments, in which the proportion of preys consumed by a predator decreases exponentially as the prey density increases, hence the linear term is negative. The logistic regression model thus can be recommended as a tool for further analyzing functional response curves. The type II and III functional responses are common among arthropod predators (Hassell et al., 1977). The results of this study are consistent with the results of Javadi and Sahragard (2005), who reported that the functional response of *A. spinidensis* on various densities of *N. aenescens* was type II. Similarly, this type of functional response has also been reported for coccinellid

**Table 1** Estimation of LC<sub>30</sub>, their confidence limit and Log dose probit-mortality data for three insecticides on *A. spinidensis*.

Insecticide	$N^a$	LC <sub>30</sub> <sup>b</sup> (95% confidence limit)	Slope ± SE	$\chi^2$ (df)
Diazinon	40	1343.51(1132.4–1515.5)	4.35 ± 0.55	1.84(5)
Fenitrothion	40	287.66(243.5–324.5)	4.33 ± 0.52	2.21(5)
Chlorpyrifos	40	312.7(250.7–365)	3.33 ± 0.44	3.31(5)

<sup>a</sup> Total number of bugs tested.

<sup>b</sup> LC<sub>30</sub> value according to ppm a.i., and 95% confidence limit.

**Table 2** Maximum likelihood estimate from logistic regression of proportion of *C. suppressalis* eaten by *A. spinidens*.

Treatment	Parameters	Estimate	SE	$\chi^2$	<i>p</i>
Control	Constant	2.3126	0.6294	13.5	0.0002
	Linear	-0.3370	0.0940	12.84	0.0003
	Quadratic	0.0102	0.00352	8.46	0.0036
	Cubic	-0.00009	0.000035	7.32	0.0068
Diazinon	Constant	1.0364	0.5521	3.52	0.0605
	Linear	-0.2320	0.0878	6.98	0.0083
	Quadratic	0.00709	0.00337	4.42	0.0354
	Cubic	-0.00007	0.000033	3.86	0.0495
Fenitrothion	Constant	0.2915	0.5490	0.28	0.5954
	Linear	-0.1587	0.0886	3.21	0.0731
	Quadratic	0.00451	0.00343	1.73	0.1882
	Cubic	-0.00004	0.00034	1.44	0.2295
Chlorpyrifos	Constant	0.8446	0.5560	2.31	0.1287
	Linear	-0.2334	0.0911	6.56	0.0104
	Quadratic	0.00673	0.00355	3.59	0.0580
	Cubic	-0.00006	0.000035	2.9	0.0886

**Table 3** Coefficient of searching efficiency (*a'*) and handling time (*T<sub>h</sub>*) (estimated by Rogers random attack equation) of *A. spinidens*.

Treatment	Parameters	Estimate	SE	95% CI	
				Lower	Upper
Control	<i>a'</i> (h <sup>-1</sup> )	0.0495	0.0155	0.0176	0.0813
	<i>T<sub>h</sub></i> (h)	2.3193	0.2808	1.8160	2.9666
Diazinon	<i>a'</i> (h <sup>-1</sup> )	0.0304	0.00916	0.0117	0.0492
	<i>T<sub>h</sub></i> (h)	2.5778	0.3712	1.8174	3.3382
Fenitrothion	<i>a'</i> (h <sup>-1</sup> )	0.0231	0.00786	0.00701	0.0392
	<i>T<sub>h</sub></i> (h)	2.8947	0.5201	1.8293	3.9602
Chlorpyrifos	<i>a'</i> (h <sup>-1</sup> )	0.0297	0.0118	0.00553	0.0538
	<i>T<sub>h</sub></i> (h)	3.9709	0.6218	2.6973	5.2445

*T<sub>h</sub>* – handling time; *a* – maximum attack rates; SE – standard error

predators like; *Cheilomenes sexmaculata* Fabricius, *Propylea dissecta* Mulsant, *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae) (Pervez and Omkar, 2005) and *Rhynocoris marginatus* Fabricius (Hem.: Reduviidae) (Ambrose et al., 2010). Comparison of functional response curves revealed that the three tested insecticides markedly decreased the mean of prey consumed by *A. spinidens*. Among them, functional response curve of *A. spinidens* in chlorpyrifos treatment was significantly lower than the other treatments (Fig. 1). Rezac et al. (2010) reported the spider *Philodromus cespitum* Walckenaer (Araneae: Philodromidae) exposed to NeemAzal® and Dimilin® had a significantly lower asymptote compared to the control, which showed a reduction by about one half.

Functional response manifests two important parameters including *a'* and *T<sub>h</sub>* used to evaluate the effectiveness of predators and parasitoids (Hassel and Waage, 1984). The rate of these parameters and the type of functional response in predators 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, sublethal effects of diazinon, fenitrothion and chlorpyrifos on functional response parameters of *A. spinidens* showed different searching

efficiency and handling time compared to control. Our findings demonstrated that the bugs exposed to insecticides had a higher handling time (Table 3), in which the highest *T<sub>h</sub>* occurred in the chlorpyrifos treatment. Moreover, effects of insecticides on *a'* revealed that the value of this parameter decreased with insecticide application and the lowest value was observed in the bugs exposed to fenitrothion (0.0231 ± 0.00786). The attack rate or instantaneous rate of discovery or searching efficiency (*a'*) is the proportion of the total area searched by a predator/unit of searching time. It determines how rapidly the functional response curve approaches the upper plateau. Moreover, it is a function of (1) maximum distance at which the predator can perceive the prey, (2) speed of movement of predator that can perceive the prey, (3) proportion of attacks that are successful (Holling, 1965, 1966). Li et al. (2006) reported that acarophagous thrips, *Scolothrips takahashii* Priesner (Thysanoptera: Thripidae) females exposed to mancozeb showed prolonged handling time. Similarly, fenpropathrin and abamectin caused significantly lower attack rates and prolonged handling time in both males and females. Perturbation of predation by pesticides may drastically reduce the efficiency of natural enemies.

Pesticides used in this study were organophosphorus (OP) insecticides that 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). For example, the insecticide chlorpyrifos used for the control of the coffee leaf miner *Leucoptera coffeella* Guérin-Méneville (Lep.: Lyonetiidae) was not selective to vespoid predators in coffee plantations. Galvan et al. (2002) found similar results for wasps, *Protonectarina sylveirae* Saussure (Hymenoptera: Vespidae), *Brachygastra lecheguana* Perty (Hym.: Vespidae) and *P. exigua* de Saussure (Hym.: Vespidae) for the insecticide fenitrothion. The highest toxicity of the organophosphates to predators may be associated with the pro-insecticide activity of this group. When these compounds penetrate organisms, they suffer reactions and become more toxic. Another factor possibly related to the toxicity of organophosphates is the lipophilic characteristic of some insecticides associated with thickness and lipid composition of the insect cuticle. Such relation is accountable for the penetration of the product into the insect cuticle and the translocation to target of site (Fernandes et al., 2010). Organophosphates are highly lipid-soluble agents and absorbable, therefore, in this study when insecticides were topically applied on the thoracic dorsum of *A. spinidens* nymphs they could be easily absorbed and translocated to the target site. This might have been the reason for high toxicity of used compounds. On the other hand, the basic mechanism of action of organophosphate insecticides is based on acetylcholinesterase (AChE) inhibition. They are subsequently accumulated in neuromediator acetylcholine at the cholinergic synapses, either peripheral or central. They could cause cholinergic hyper stimulation and development of symptoms of poisoning such as hyperexcitation, restlessness, incoordination, prostration and paralysis. It is reported that the behavioral alterations in motility of natural enemy include lack of motor coordination, tremors, downfalls, abdomen tucking and rotational movement for abdomen cleaning may increase after application of insecticides (Suchail et al., 2001). In the present study we observed leg and proboscis tremor on *A. spinidens* after pesticide treatment, within minutes to hours. This case may affect the feeding behavior



of exposed predators and explain the reduced capture of *A. spinidensis* in pesticide treatments compared with the control. In addition, as the abnormal behavior increased, the time that takes for a predator to encounter and consume a single prey may be prolonged. According to the handling time, time-consuming activities may prolong handling time and cause delayed predatory acts. Deng et al. (2007) found that contact with organophosphorus compounds resulted in increased handling time in *Hylyphantus graminicola* Sundevall (Araneae: Linyphiidae). A similar observation was reported by Ambrose and George (1998) in monocrotophos-treated *Acanthaspis pedestris* Stal (Hem.: Reduviidae). The negative effects of insecticides on functional response have been reported in many natural enemies. Ambrose et al. (2010) studied the impact of Synergy-505® (chlorpyrifos 50% and cypermethrin 5% E.C), on functional response of *R. marginatus* and reported that this chemical compound caused a less pronounced type II functional response with reduced numbers of prey killed, attack rate, searching time, and prolonged handling time in 4th and 5th nymphal instars, adult males and females reflecting reduced predatory potential. Rafiee-Dastjerdi et al. (2009) demonstrated that profenofos, thiodicarb, hexaflumuron and spinosad had a negative effect on functional response of *Habrobracon hebetor* Say (Hym.: Braconidae). They reported that the wasps exposed to insecticides had higher handling time and among the pesticides, spinosad caused the most negative effect on searching efficiency. In addition, Abedi et al. (2012) showed that the application of cypermethrin on *H. hebetor* caused longest handling time and lowest attack rate. In other study, the functional response of the spider, *P. cespitum* was significantly affected after application of SpinTor®, NeemAzal®, and Dimilin® (Rezac et al. 2010). Furthermore, the effect of cypermethrin on the functional response of *A. pedestris* indicated that the pesticide negatively affected the functional response events such as attack ratio, handling time and rate of discovery and also reduced the predatory efficiency (Claver et al., 2003). However, most of the studies reported negative effects of insecticides on the functional response of natural enemies. However, a positive effect on predation rate has been observed for some low-dose of pesticides. For example, a stronger functional response was seen in wolf spiders, *Pardosa pseudoannulata* Boes. and Strand (Wang et al., 2006) and increased consumption was seen in *Mallada signatus* Schneider (Neuroptera: Chrysopidae) (Qi et al., 2001). These results can be explained by the general theory of hormoligosis (the stimulation of reproductive physiology by sublethal doses of pesticides) (Luckey 1968; Stebbing, 1982) or by the disruption of predator avoidance ability of prey.

## 5. Conclusion

In an integrated control program, it is necessary to utilize some insecticides with minimal toxicity to natural enemies of pests. In this study, the sublethal effects of diazinon, fenitrothion and chlorpyrifos were evaluated on the functional response of *A. spinidensis*. These pesticides are organophosphate compounds that are widely used for controlling rice lepidopterous pests in north of Iran. Among the pesticides, fenitrothion and chlorpyrifos caused considerable effects on the functional response of *A. spinidensis* nymphs compared to diazinon pesticide. The results showed that the attack rate and handling time in

exposed bugs were affected by fenitrothion and chlorpyrifos more than diazinon. Diazinon is commonly used to control pests in all rice fields of Iran. Since, the pesticide has extensive use compared to two other pesticides it seems that bugs exposed to diazinon are more compatible with this pesticide. In addition, data obtained from LC<sub>30</sub> values confirmed low toxicity of this pesticide on *A. spinidensis* nymphs compared with other pesticide treatments. In the present study, although the used concentrations were lower than the recommended field rate for rice pest control, our results indicated that these concentrations had adverse effects on functional response of the predatory bug. According to our finding, diazinon, fenitrothion and chlorpyrifos are not suitable for use in the IPM of rice pests with *A. spinidensis*. These insecticides markedly reduced the functional response of *A. spinidensis* and potentially limited its biocontrol potentiality. It is recommended that where the use of these pesticides is essential for control of rice pests, they should be used by caution. In this way, at times of the highest population of *A. spinidensis* in the rice field in June and July, the extensive use of pesticides should be avoided.

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