

Toxicity of some biorational and conventional insecticides to cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its ectoparasitoid, *Habrobracon hebetor* (Hymenoptera: Braconidae)

H. Rafiee Dastjerdi^{1&2}, M. J. Hejazi^{1&*}, G. Nouri Ganbalani² and M. Saber³

1. Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, 51666-14888, Iran, E-mail: h_rafiee@tabrizu.ac.ir, 2. Department of Plant Protection, Faculty of Agriculture, University of Mohaghegh Ardabili, Ardabil, 55181-83111, Iran, E-mail: gadimouri@yahoo.com, 3. Department of Plant Protection, Faculty of Agriculture, Higher Education Institute of Maragheh, Maragheh, 56199-11367, Iran, E-mail: moosaber@yahoo.com

*Corresponding author, E-mail: mjhejazi@tabrizu.ac.ir

Abstract

Insect growth regulators and spinosyns which are physiologically and ecologically selective, respectively, have been investigated as replacements or complements to non-selective conventional insecticides. The effects of diflubenzuron, hexaflumuron, profenofos, spinosad and thiodicarb were assessed on the 1st instars of cotton bollworm, *Helicoverpa armigera* (Hübner) using dietary and leaf disc bioassay methods. Based on modes of action of the insecticides tested, the mortalities were recorded after 24 h in profenofos, thiodicarb and spinosad experiments and after 120 h in hexaflumuron and diflubenzuron treatments. The LC₅₀ values for diflubenzuron, hexaflumuron, profenofos, spinosad and thiodicarb in dietary method were 595.05, 0.31, 3.69, 0.13 and 11.2 mg ai/L; and in leaf disc method, they were >2000, 0.46, 9.55, 0.2 and 15.52 mg ai/L, respectively. The effects of these insecticides on adult *Habrobracon hebetor* Say, an ectoparasitoid of cotton bollworm were tested using residual method. The mortalities were recorded after 24 h in all treatments. The LC₅₀ values for diflubenzuron, hexaflumuron, profenofos, spinosad and thiodicarb for females were >2000, >2000, 12.44, 15.64 and 81.04 mg ai/L, respectively and for males, they were >2000, >2000, 6.91, 11.73 and 40.39 mg ai/L, respectively. In this study spinosad and hexaflumuron seemed to be more useful than the other insecticides due to their higher toxicity to *H. armigera* and lower toxicity to *H. hebetor*.

Key words: Organophosphate, carbamate, chitin synthesis inhibitors, biocontrol agent

چکیده

پژوهشگران مختلف، تنظیم کننده های رشد حشرات و اسپاینوزین ها را، که به ترتیب از نظر فیزیولوژیکی و اکولوژیکی انتخابی می باشند، به عنوان جایگزین یا مکمل حشره کش های قدیمی تر غیرانتخابی مورد بررسی قرار داده اند. اثرات دایفلوبنزوران، هگزافلوموران، پروفنوفوس، اسپاینوسد و تیودیکارب روی لاروهای سن اول کرم قوزی پنبه *Helicoverpa armigera* (Hübner) به روش های اختلاط با غذای مصنوعی و دیسک برگگی مورد بررسی قرار گرفتند. با توجه به نحوه عمل حشره کش های مورد بررسی، مرگ و میر در تیمارهای پروفنوفوس، تیودیکارب و اسپاینوسد بعد از ۲۴ ساعت و در هگزافلوموران و دایفلوبنزوران بعد از ۱۲۰ ساعت ثبت گردید. مقادیر LC₅₀ برای دایفلوبنزوران، هگزافلوموران، پروفنوفوس، اسپاینوسد و تیودیکارب در روش غذای مصنوعی به ترتیب ۵۹۵/۰۵، ۰/۳۱، ۳/۶۹، ۰/۱۳ و ۱۱/۲ و در روش دیسک برگگی به ترتیب >۲۰۰۰، ۰/۴۶، ۹/۵۵، ۰/۲ و ۱۵/۵۲ میلی گرم ماده ی مؤثر در لیتر بود. این حشره کش ها همچنین روی حشرات کامل اکتوپارازیتوئید *Habrobracon hebetor* Say با استفاده از روش سطح آغشته به حشره کش آزمایش شدند. در این بررسی مرگ و میر در همه تیمارها بعد از ۲۴ ساعت ثبت شد. مقادیر LC₅₀ برای دایفلوبنزوران، هگزافلوموران، پروفنوفوس، اسپاینوسد و تیودیکارب به ترتیب برای حشرات ماده >۲۰۰۰، >۲۰۰۰، ۱۲/۴۴، ۱۵/۶۴ و ۸۱/۰۴ و برای نرها به ترتیب >۲۰۰۰، >۲۰۰۰، ۶/۹۱، ۱۱/۷۳ و ۴۰/۳۹ میلی گرم ماده ی مؤثر در لیتر بود. در این تحقیق، اسپاینوسد و هگزافلوموران به دلیل سمیت بیشتر روی آفت و صدمه ی کمتر به اکتوپارازیتوئید مفیدتر از سایر حشره کش های مورد بررسی بودند.

واژگان کلیدی: ترکیبات آلی فسفره، کاربامات ها، مهارکننده های ساخت کتین، عوامل کنترل زیستی

Introduction

Cotton bollworm, *Helicoverpa armigera* (Hübner), is a polyphagous agricultural pest which attacks a wide variety of agricultural crops including cotton, corn, tomatoes, sorghum, soybeans and groundnuts (Fitt, 1989; Matthews, 1999). Early instars are foliar feeders and later instars attack seeds, fruits and bolls leading to economic loss (Fitt, 1989). Presently chemical control of *H. armigera* in Iran is mainly done using endosulfan, profenofos and thiodicarb (Mosallanazhad *et al.*, 2003). Conventional chemical insecticides such as organophosphates (OPs), carbamates and pyrethroids have caused development of resistance, resurgence and outbreaks in *H. armigera* populations in several countries such as Australia, Pakistan and Palestine. The deleterious effects of insecticides on natural enemies are among the major causes of pest outbreaks (Gunning *et al.*, 1984; Horowitz *et al.*, 1993; Ahmad *et al.*, 2001). *Habrobracon hebetor* Say is a valuable biocontrol agent of lepidopteran pests attacking crop plants and stored products, including *H. armigera* (Brower & Press, 1990; Magro & Para, 2001). In Iran, mass rearing of *H. hebetor* is done on Mediterranean flour moth, *Anagasta kuehniella* (Zeller) and the adult wasps are released to parasitize *H. armigera* larvae in cotton fields in Ardabil and Golestan provinces in northern parts of the country (Attaran, 1996; Navaei *et al.*, 2002).

Most of the insecticides currently used against agricultural pests are neurotoxic compounds such as organophosphates and carbamates that result in relatively inexpensive and reliable control (Haynes, 1988). Development of resistance to neurotoxic insecticides in key pests has led to restrictions in the use of these insecticides (Biddinger & Hull, 1995; Cox *et al.*, 1995). Many broad-spectrum insecticides, especially OPs are very toxic to natural enemies (Bayoun *et al.*, 1995; Legaspi *et al.*, 1999). Natural enemies are usually more susceptible to insecticides than their hosts (Croft, 1990). Wilson *et al.* (1999) reported that a higher number of aphids on cotton plants sprayed with thiodicarb were due to suppression of predators. The efficiency of natural enemies is reduced by application of most conventional insecticides (O'Brien *et al.*, 1985). However, some natural enemies can become resistant to them (Croft, 1990).

Benzoylphenylureas (BPU) are biorational insecticides that disrupt the insect biochemistry and physiology (Retnakaran *et al.*, 1985). They may also cause moulting disorder and insect sterility (Hejazi & Granett, 1986a, b). Insect growth regulators (IGRs) and spinosyns which are physiologically and ecologically selective, respectively, have been investigated as replacements or complements to the OP and carbamate insecticides. These insecticides are less toxic to biocontrol agents than conventional insecticides such as OPs and

carbamates (Biddinger & Hull, 1995). The IGRs are highly selective to insects and take more time to reduce insect populations than conventional insecticides (Dent, 2000). They could further facilitate biologically based pest management in cotton production systems (Naranjo *et al.*, 2004). These compounds may be good alternatives for conventional insecticides for a selective insect pest control (Tunaz & Uygun, 2004). Restricting the use of insecticides and preserving natural enemies are important in IPM programs (Horowitz *et al.*, 1993). To get the best combination of natural enemies and insecticides, evaluating the effects of various insecticides on natural enemies is essential (Hsieh & Allen, 1986; Croft, 1990; Dent, 1995). The potential of biological control to contribute to pest suppression in managing agricultural systems is limited by the use of insecticides with broad toxicity to both pests and their natural enemies (Croft, 1990). There is less knowledge about the effects of chemical insecticides on predators and parasites compared with pest arthropods (Croft, 1990). In the current study, lethal effects of some conventional and biorational insecticides were assessed on *H. armigera* and its ectoparasitoid, *H. hebetor*.

Materials and methods

Insects

The larvae of *H. armigera* were originally collected from Parsabad, a town 232 Km north of Ardabil located in Ardabil province of Iran and reared on modified Shorey & Hale's pinto bean-based artificial diet (Shorey & Hale, 1965) in the greenhouse for 12 generations prior to use in bioassays. Adults of *H. hebetor* were obtained from an insectarium maintained by Plant Protection Bureau of Bilehsavar in Ardabil province and reared on 5th instars of *A. kuehniella* in the laboratory. Up to 24-h-old 1st instars of cotton bollworm and up to 48-h-old adults of *H. hebetor* were used in the experiments. Rearing conditions were 26 ± 2 °C, $70 \pm 5\%$ RH, and photoperiod of 16: 8 h (L: D).

Insecticides

Insecticides tested were diflubenzuron (25 WP, Hebei Vian Bio-chemical, <http://www.veyong.com/english/Product.asp>); hexaflumuron (Consult[®] 10 EC) and spinosad (SpinTor[®] 25 SC) (both from Dow AgroSciences, <http://dowagro.com/uk/products>); profenofos (40 EC, Golsam, <http://www.golsam.com>); and thiodicarb (80 DF, Gyah, <http://www.gyah.ir>).

Bioassays - cotton bollworm

In this study, the toxicities of the insecticides were assessed on 1st instars of *H. armigera* using dietary and leaf disc methods. The ranges of concentrations for different insecticides were determined by preliminary dose setting experiments. In dietary method, 1 ml from each concentration of the insecticides was incorporated into 9 ml of the artificial diet in 100 ml glass containers. The actual (after incorporation of the insecticides into diet) concentration ranges for diflubenzuron, hexaflumuron, profenofos, spinosad and thiodicarb in dietary method were 300-1000, 0.25-0.5, 2-8, 0.08-0.2 and 5-15 mg ai/L, respectively; and those for hexaflumuron, profenofos, spinosad and thiodicarb in leaf disc method were 0.25-0.7, 5-20, 0.16-0.3 and 10-50 mg ai/L, respectively. First instars of *H. armigera* were transferred on the treated diet in the containers. In leaf disc experiments the leaves from Acala cotton cultivar were used. Leaf discs 5 cm in diameter were dipped into different concentrations of the insecticides for 10 seconds and let dry for 15 minutes. The 1st instars of *H. armigera* were then transferred on the leaf discs placed in 100 ml glass containers. Up to 15 first instars of *H. armigera* were put in each treatment in both methods. Based on the modes of action of the insecticides tested, the mortalities were recorded after 24 h in profenofos, thiodicarb and spinosad experiments and after 120 h in hexaflumuron and diflubenzuron treatments.

Bioassays - ectoparasitoid

Male and female adults of *H. hebetor* were used for bioassays using Potter Spray Tower (Burkard, U.K., www.burkardscientific.co.uk/agronomics/spray_tower.htm). Exposure cages were used for experiments. The exposure cages consisted of two 100 × 100 mm glass plates which covered a 10 mm thick polyethylene frame of the same size (100 × 100 mm) and acted as floor and ceiling for the cage. Two sides of the frame consisted of four ventilation holes 5 mm in diameter, covered with 40 mesh cloth. Both inner surfaces of the glass plates were sprayed with 2 ml of aqueous solution of each insecticide concentration (Saber *et al.*, 2005). The concentration ranges for profenofos, spinosad and thiodicarb for females were 5-50, 5-75 and 50-250 mg ai/L, respectively, and for males, they were 3-18, 5-50 and 20-80 mg ai/L, respectively. Triton X 100 was used as the wetting agent at a concentration of 555 ppm in this experiment. The control plates were sprayed with distilled water plus Triton X-100. The operating pressure was 0.5 bar and the mean spray deposit was $1.68 \pm 0.04 \mu\text{l}/\text{cm}^2$. After drying of the glass plates at room temperature and assembling of the exposure cages, the adult wasps were transferred to the cages. Honey was supplied as food for the adult parasitoids on 5 × 30 mm strips of paper placed in the cages. Up to 20 adults of *H. hebetor* were used in each

treatment. The mortalities were recorded after 24 h in all treatments. Five concentrations of each chemical and three to five replicates at different days were used in all experiments including cotton bollworm and the ectoparasitoid.

Data analysis

The data were analyzed using the probit procedures with SAS for Windows® release 9.0 (SAS Institute, 2002). To compare toxicity of the same insecticide in different bioassay methods, as well as the toxicity of different chemicals with each other, the ratios of the LC₅₀ values and their related 95% confidence limits were calculated (Robertson & Preisler, 1992).

Results and discussion

The results of assessing the toxicities of various insecticides on 1st instar *H. armigera* in dietary and leaf disc methods are shown in tables 1 and 2. In both methods toxicities of the insecticides tested were significantly different.

Table 1. Toxicity of the insecticides tested on first instar *H. armigera* in dietary method.

Insecticide	Category	n	Slope ± SE	LC ₅₀ (mg ai/L) (95% CL)	LC ₉₀ (mg ai/L) (95% CL)	χ ²
Diflubenzuron	Chitin synthesis inhibitor	480	4.18 ± 0.45	595.05 (547.66 – 647.25)	1205.06 (1040.47 – 1495.30)	2.25
Hexaflumuron	Chitin synthesis inhibitor	450	4.10 ± 0.72	0.31 (0.27 – 0.34)	0.64 (0.54 – 0.89)	2.24
Profenofos	Organophosphate	480	3.66 ± 0.43	3.69 (3.3 – 4.07)	8.26 (7.05 – 10.48)	1.94
Spinosad	Spinosyns	435	4.57 ± 0.57	0.13 (0.12 – 0.14)	0.26 (0.23 – 0.32)	1.84
Thiodicarb	Carbamate	450	2.74 ± 0.47	11.20 (9.85 – 13.39)	32.87 (23.41 – 63.97)	0.57

Table 2. Toxicity of the insecticides tested on first instar *H. armigera* in leaf disc method.

Insecticide	Category	n	Slope ± SE	LC ₅₀ (mg ai/L) (95% CL)	LC ₉₀ (mg ai/L) (95% CL)	χ ²
Hexaflumuron	Chitin synthesis inhibitor	408	2.98 ± 0.52	0.46 (0.41 – 0.53)	1.26 (0.94 – 2.23)	0.77
Profenofos	Organophosphate	405	4.14 ± 0.43	9.55 (8.71 – 10.43)	19.48 (16.96 – 23.73)	3.26
Spinosad	Spinosyns	360	5.73 ± 0.91	0.20 (0.19 – 0.22)	0.35 (0.31 – 0.43)	0.24
Thiodicarb	Carbamate	486	3.96 ± 0.38	15.52 (13.98 – 17)	32.67 (28.91 – 38.50)	1.04

Diflubenzuron concentrations as high as 2000 mg ai/L did not result in considerable mortality. Hence, its LC values were not estimated.

Spinosad was the most toxic of the insecticides tested. Hexaflumuron, a chitin synthesis inhibitor (CSI) was the second most toxic insecticide in both methods. On the contrary, diflubenzuron, which also is a CSI, was more than 1900 times less toxic to *H. armigera* in dietary method than hexaflumuron. The second generation acylureas have the greater insecticidal activity compared with diflubenzuron. Also wettable powder (WP) formulations of these compounds have relatively poor performance compared with emulsifiable concentrates (ECs) (Ismail *et al.*, 1992). Diflubenzuron concentrations as high as 2000 mg ai/L did not result in considerable mortality in the leaf disc method. Hence, its LC values could not be estimated. Profenofos was 3 times more toxic to 1st instar cotton bollworm than thiodicarb in dietary method and 1.6 times more toxic in leaf disc method (tables 1 and 2). The trend of toxicity of different compounds was similar in both methods, but the LC₅₀ values in leaf disc method were 1.5-2.6 times more than the dietary method. These differences in toxicities of the different chemicals in the two bioassay methods were significant ($P < 0.05$). This could have been due to the difference in the amount of the toxicant available to larvae in the two methods. In leaf disc method only the surfaces of the leaf discs were impregnated with the insecticides, while in dietary method; the insecticides were completely incorporated into artificial diet. The results of residual toxicity of selected insecticides to adults of *H. hebetor* are shown in tables 3 and 4.

Table 3. Toxicity of the insecticides tested on females of *H. hebetor* in residual method.

Insecticide	Category	n	Slope ± SE	LC ₅₀ (mg ai/L) (95% CL)	LC ₉₀ (mg ai/L) (95% CL)	χ ²
Profenofos	Organophosphate	389	2.20 ± 0.24	12.44 (10.46 – 14.59)	47.34 (36.58 – 68.81)	2.11
Spinosad	Spinosyns	440	1.37 ± 0.17	15.64 (12.08 – 19.71)	134.55 (86.32 – 272.62)	1.14
Thiodicarb	Carbamate	360	3.54 ± 0.43	81.04 (71.74 – 90.04)	186.28 (157.76 – 239.84)	3.52

Diflubenzuron and hexaflumuron concentrations as high as 2000 mg ai/L did not result in considerable mortality. Hence, their LC values were not estimated.

Diflubenzuron and hexaflumuron concentrations as high as 2000 mg ai/L did not result in considerable mortality. Hence, their LC values were not estimated. Profenofos, spinosad and thiodicarb were more toxic to both males and females. The trend of toxicity of profenofos, spinosad and thiodicarb to both males and females was similar, but the males were more susceptible than females. This may be due to the higher amount of fat body in females than males. Also insecticide metabolism might have been higher in females than

males, because females have a higher content of cytochrome P-450 than males (Agosin, 1985). Guedes *et al.* (2006) reported that insecticide resistant population of *Sitophilus zeamais* Motschulsky had larger fat body cells than susceptible population and as a result a higher capacity for detoxification of toxic compounds. Penagos *et al.* (2005) reported that adult males of *Euplectrus plathyphenae* Howard exposed to fresh spinosad residues on maize leaves were more susceptible than female parasitoids. Based on the results obtained from the current study, toxicity of spinosad and profenofos to female *H. hebetor* was not significantly different. Spinosad was 28-47 times more toxic to 1st instar *H. armigera* and 1.7 times less toxic to male *H. hebetor* than profenofos. Also spinosad was 77-86 times and 3.44-5.18 times more toxic than thiodicarb to 1st instar *H. armigera* and adult *H. hebetor*, respectively.

Table 4. Toxicity of the insecticides tested on males of *H. hebetor* in residual method.

Insecticide	Category	n	Slope ± SE	LC ₅₀ (mg ai/L) (95% CL)	LC ₉₀ (mg ai/L) (95% CL)	χ ²
Profenofos	Organophosphate	394	2.28 ± 0.29	6.91 (5.89 – 8.05)	25.19 (18.95 – 39.68)	0.5
Spinosad	Spinosyns	457	2.66 ± 0.39	11.73 (9.45 – 13.51)	35.55 (29.29 – 49.29)	1.44
Thiodicarb	Carbamate	402	2.72 ± 0.36	40.39 (35.40 – 46.11)	119.32 (92.47 – 181.44)	0.14

Diflubenzuron and hexaflumuron concentrations as high as 2000 mg ai/L did not result in considerable mortality. Hence, their LC values were not estimated.

If similar results are obtained for these chemicals in the field, using the reduced doses in IPM context, spinosad might be a more valuable chemical to adequately control *H. armigera* while minimum harm is done to *H. hebetor*. Mendez *et al.* (2002) reported that at recommended field rate (200 ppm) spinosad caused 100% mortality of *Spodoptera frugiperda* (J. E. Smith) and 19-65% mortality in the pteromalid parasitoid, *Catolaccus grandis* (Burks). While using 3 ppm of spinosad resulted in 75-82% control of *S. frugiperda*, it had little impact on the insect natural enemies on maize plants. In the current study, it was found that diflubenzuron and hexaflumuron were less toxic to *H. hebetor* compared with profenofos, spinosad and thiodicarb. Naranjo *et al.* (2004) also reported that survival of parasitoids and rates of parasitism were higher in cotton fields sprayed with IGRs compared with those sprayed with conventional insecticides. Legaspi *et al.* (2000) studied contact toxicity of diflubenzuron on two hymenopteran parasitoids and one ladybeetle, and found this insecticide compatible with biocontrol agents. We found very low toxicity of diflubenzuron against *H. armigera* while Granett & Hejazi (1983) reported that *Spodoptera exigua* (Hübner) was

considerably more susceptible to diflubenzuron. Since, *H. hebetor* is also released to control *Spodoptera* spp. (Magro & Parra, 2001); this compound may be used against *Spodoptera* spp. with no adverse effects on *H. hebetor*. The IGRs may be considered as alternative chemicals with a high potential for controlling certain pests and less adverse effects on natural enemies.

Acknowledgments

We are grateful to S. Alijani for his help in analysis of data. This work received financial support from Postgraduate Education Bureau of the University of Tabriz which is appreciated.

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Received: 30 July 2007

Accepted: 24 December 2007