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Toxicity of chlorpyrifos, spinosad and abamectin on cotton bollworm, *Helicoverpa armigera* and their sublethal effects on fecundity and longevity

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Abstract

Cotton bollworm, *Helicoverpa armigera* Hubner is the most important pest of cotton, tomato and chickpea in Iran. The pest has been found to develop resistance against conventional insecticides. Using of insecticides with different mode of action may result an appropriate control of the pest and may delay insecticide resistance development. In this study, we attempted to investigate the lethal and sublethal effects of one conventional, two biorational insecticides on larvae at different stages of *H. armigera*, and its adult longevity and fecundity under the laboratory condition. The LC_{50} values of chlorpyrifos, spinosad and abamectin were 4.6, 62.26 and 460.5 ppm based on formulated materials. The results showed that chlorpyrifos and spinosad were more effective insecticides against 3rd instar larvae of cotton bollworm compared to abamectin based on formulated materials. Furthermore, abamectin and chlorpyrifos negatively affected longevity and fecundity of adults that emerged from treated third instars. But, spinosad had no significant effect on longevity and fecundity of the adults. All the tested insecticides seemed to be effective against cotton bollworm. Spinosad and abamectin might be preferred due to their environmental friendly impacts. Abamectin also markedly reduced female fecundity and longevity for adults exposed as third instars larvae compared with spinosad. The larvicidal and reproductive effects of abamectin against the pest and its biorationality suggest that abamectin is suitable for integration into an IPM program for cotton bollworm.

Key words: *Helicoverpa armigera*, biorational insecticides, life table parameters, lethal and sublethal.

Introduction

Cotton bollworm, *Helicoverpa armigera* Hubner is the most destructive pest of cotton and tomato in Iran. The cotton bollworm feeds on most plant parts including stem, leave, flower buds, flower heads and fruits at different larval development stages (Moral Garcia, 2006). This pest can survive in unstable habitats and colonize various agricultural ecosystems (Fitt, 1989). This pest with characteristics such as, ability to migrate, high fecundity, facultative diapause, polyphagy and ability to develop resistance to conventional insecticides causes considerable damages on the host plants (Fitt, 1989; MacCaffery, 1998). In Iran, the damage caused by the pest is around 20-25% of the yield and rises to 50-75% at times of outbreak of the pest (Mojeni *et al.*, 2005). Similar to most of other cotton and tomato growing countries, insecticides are largely used for controlling cotton bollworm in Iran. Endosulfan, profenofos and thiodicarb have been the commonly used insecticides for controlling *H. armigera* in recent years in Iran (Mosallanazhad *et al*., 2003). The use of widespread insecticides at high doses, as well as inappropriate timing have cause adverse effects on natural enemies and resulte in resurgence of cotton bollworm (Metcalf and Luckman, 1975). The pest has developed resistance against conventional insecticides such as chlorinated hydrocarbons, synthetic pyrethroides and organophosphates in Pakistan (Ahmad *et al*., 1998a, 1998b, Ahmad *et al*., 1999), Spain (Torres-Vila *et al.*, 2002a; 2002b) and Australia (Gunning *et al*., 1984; Gunning and Easton, 1994). Despite no record of resistance of cotton bollworm against insecticides in Iran, establishment of resistance to conventional insecticides by this pest is not unexpected.

Therefore, using insecticides with different mode of action may result an appropriate control of the pest and may delay insecticide resistance development. Chlorpyrifos, spinosad and abamectin were selected for studying against *H. armigera* from different classes of the insecticides. Chlorpyrifos exerts its toxic action by inhibiting certain important enzymes of the nervous system, cholinsterases (ChE) (Ware and Whitacre, 2004). Mode of action of abamectin is blocking of the neurotransmitter gamma aminobutyric acid (GABA), at the neuromuscular junction in insects and mites (Ware and Whitacre, 2004). Spinosad acts by disrupting binding of acetylcholine to nicotinic acetylcholine receptors at the postsynaptic cells (Salgado, 1997). Field studies were carried out to evaluate the efficacy of different insecticides such as chlorpyrifos, endosulfan, profenofos and spinosad against lentil pod borer (*Helicoverpa* spp.) and results showed that spinosad was the most effective insecticide in reducing the population of lentil pod borer (Ahamad Memon *et al*., 2005).

Nirmal and Manjit (2008) reported that spinosad and chlorpyrifos had higher efficacy against third instar larvae of cotton bollworm compared with endosulfan, acephate and cypermetrin. Abamectin and spinosad are biorational insecticides that are known as antibiotics (Ware and Whitacre, 2004). Abamectin is produced by the soil bacterium, *Streptomyces avermitilis* Burg (White *et al*., 1997). Spinosad is derived from soil actinomycetes, *Saccharopolyspora spinosa* Mertz et Yao, containing a naturally occurring mixture of spinosyn A and spinosyn D. Spinosad had high toxicity to *H. armigera* and was a safe insecticide to some of natural enemies (Rafiee Dastjerdi *et al.*, 2008; Amalendu, 2010). But it seems that sublethal doses of pesticides may affect physiology and behavior of pests and theirs natural enemies (Johnson and Tabashnik, 1999). It has been reported that sublethal doses of insecticides may reduce and or increase longevity, fertility or fecundity of pests. Therefore, the study of sublethal effects of insecticides may show ecological aspects of insecticide applications and provides a suitable pest management program. In this study, we investigated the effects of lethal and sublethal effects of two biorational and one conventional insecticide on longevity and fecundity of an important insect pest of cotton and tomato *H. armigera* under laboratory conditions.

Materials and methods

Insect

The eggs of cotton bollworm were taken from Department of Plant Protection of the University of Tabriz, Iran. When the eggs were hatched, the larvae were transferred to plastic containers containing artificial diet. The artificial diet was made using cowpea powder 205 gr, powdered agar 14 g, ascorbic acid 3.5 g, sorbic acid 1.1 g, methyl-p-hydroxybenzoate 2.2 g, yeast 35 g, wheat germ 30 g, formaldehyde 37% 2.5 ml, vegetable oil 5 ml and distilled water 650 ml (Shorey and Hale, 1965). In order to prevent cannibalism, the larvae were individualy transferred into a 33 ml glass vials at third instar. The vials were maintained in an insectarium at 25 ± 1 °C, 70 ± 5% RH and a photoperiod of 16:8 (L:D) until pupation. After emergence, 15 pairs of adult moths were released into 18× 20 cm plastic container with 1:1 sex ratio for mating and egg-laying. The adults were fed on a 10% honey solution.

Insecticides

Insecticides tested were spinosad (SpinTor® 24SC, Dow AgroSciences, India), chlorpyrifos (Dursban® 48EC, DowElanco, England) and abamectin (Gyamectin® 1.8EC, Gyah, Iran).

Bioassay

The toxicity of insecticides was assessed on 3rd instar larvae of the cotton bollworm. Third instar larvae of the pest were exposed to spinosad by mixing the insecticide dilutions with artificial diet and exposed to abamectin and chlorpyrifos by residue contact method. Oral bioassay method was chosen for spinosad because although it exhibits good activity via topical or contact application, better activity is observed by oral application or by injection (Sparks *et al.*, 1997; 1998) suggesting that penetration through the insect cuticle may be comparatively slow (Salgado and Sparks, 2010). The ranges of concentrations were determined for the insecticides by preliminary dose-setting tests. The ranges of used concentrations were 30-100, 3-7, and 200-800 ppm for spinosad, chlorpyrifos and abamectin, respectively. The main bioassay tests were conducted with six different concentrations based on logarithmic intervals for each insecticide. Each concentration involved 3 replications and each bioassay test was replicated three times. After applying the insecticides, 20 third instar larvae were transferred into Petri dishes (9 cm diameter) for each concentration in both methods. The Petri dishes were kept in incubator at 25 ± 1 °C, 70 ± 5% RH and a photoperiod of 16:8 (L:D). Mortality was recorded after 24 h in all experiments.

Determination of sublethal effects of *H. armigera*

About 100 third instar larvae of cotton bollworm were treated with LC_{30} of each insecticide by the same methods used for bioassay tests for them. After 24 hours, the survivors were collected and kept in glass vials individually on artificial diet until pupation. The pupal weight and life span of pupal were recorded. For fecundity and longevity study, each pair of emerging male and female adults were collected and kept in cylindrical containers. The adults were fed on 10% honey solution. The experiments were carried out with 10 replications for all tested insecticides and control. The number of eggs laid by each female was recorded daily until the female died. The longevity of female adult was recorded as well. Experiment were conducted at 25 ± 1 °C, 70 ± 5% RH and a photoperiod of 16:8 (L:D).

Statistical analysis

The data were analyzed using probit procedures of SAS program (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 LC_{50} values and their related 95% confidence limits were calculated (Robertson and Preisler, 1992).

Results

Bioassays

The LC_{90}, LC_{50} and LC_{30} values of the tested insecticides against third instar larvae of *H. armigera* are presented in table 1. Based on LC_{50} values, chlorpyrifos was the most toxic insecticide on third instar larvae of cotton bollworm followed by spinosad and abamectin. The toxicity of insecticides tested was significantly different (table 1).

Sublethal effects on pupae

Sublethal effects of LC_{30} of the tested insecticides on pupal weight and period are shown in table 2. The pupal periods and pupal weight of cotton bollworm in treatment with abamectin and chlorpyrifos were significantly the longer and lighter than the control (pupal periods; $F = 11.4$, $df = 3$, $P < 0.0001$: pupal weight; $F = 4.4$, $df = 3$, $P = 0.01$). However, spinosad did not affect the pupal period and pupal weight significantly.

Sublethal effects on longevity and fecundity of adults

Sublethal effects of LC_{30} of spinosad, chlorpyrifos and abamectin on longevity and fecundity of female cotton

Table 1. Toxicity of spinosad, chlorpyrifos and abamectin to third instar larvae of *H. armigera*. Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute, 2002).

Insecticide	n	χ^2	Slope ± SE	Lethal concentrations (mg litre^{-1}) or [mg ai litre^{-1}] LC_{30} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Spinosad	420	65.3	3.8 ± 0.4	45.53(39.9-50.2) [10.47]	62.26(56.8-68.6) [14.31]	133.72(111.7-177) [30.75]
Chlorpyrifos	420	95.5	6.47 ± 0.6	3.81(3.5-4) [1.82]	4.6(4.3- 4.8) [2.2]	7.25(6.6-8.1) [3.45]
Abamectin	420	52.8	2.8 ± 0.3	301.45(250-344.1) [5.42]	460.45(08.4-525.) [8.28]	1296(1000 - 2001) [23.32]

Table 2. Sublethal effects of LC_{30} values of spinosad, chlorpyrifos and abamectin on pupal weight and pupal period of *H. armigera*.

Insecticide	Concentration (μg ml^{-1})	Pupal weight ± SE (mg)	Pupal period ± SE (day)
Spinosad	45.53	337.1 ± 11.2 ab	12.5 ± 0.2 b
Chlorpyrifos	3.81	312.8 ± 8 b	14.2 ± 0.4 a
Abamectin	301.45	307.5 ± 13.8 b	14.4 ± 0.3 a
Control	-	354.2 ± 3.6 a	12.1 ± 0.3 b

Means within a column followed by different letters are significantly different, Fisher protected least significant difference (LSD), P = 0.05.

Table 3. Sublethal effects of LC_{30} values of spinosad, chlorpyrifos and abamectin on longevity and fecundity of *H. armigera*.

Insecticide	Concentration (ppm)	Mean eggs per female (M_x) ± SE	Longevity (day) ± SE	Mean reduction in fecundity rate (%)	Mean reduction in longevity rate (%)
Spinosad	45.53	1299.5 ± 73.8 a	17.8 ± 0.5 a	9.9	0
Chlorpyrifos	3.81	940.2 ± 128.1 b	14.5 ± 1 b	34.8	18.5
Abamectin	301.45	916.2 ± 105.9 b	14.6 ± 1.2 b	36.5	17.9
Control	-	1443.3 ± 117.4 a	17.8 ± 1.1 a	-	-

Means within a column followed by different letters are significantly different, Fisher protected least significant difference (LSD), P = 0.05.

bollworm treated with LC_{30} of the insecticides at 3rd instar larvae are shown in table 3. Abamectin and chloryrifos reduced the mean number of eggs oviposited by cotton bollworm female significantly compared to control and spinosad (F = 6.02; df = 3; P = 0.001). Female fecundity was reduced by 36.5, 34.5 and 9.9% in abamectin, chloryrifos and spinosad treatments compared to control, respectively.

Treatments of third instar larvae with abamectin and chlorpyrifos reduced longevity of adults significantly (F = 3.84; df = 3; P = 0.015). However, spinosad did not affect adult longevity, significantly. Abamectin and chlorpyrifos reduced the longevity by 18.5 and 17.5% compared with control, respectively. There were non-significant differences between abamectin and chlorpyrifos effects on adult longevity. And also adult longevity was not affected significantly by spinosad.

Sublethal effect on adult oviposition

Oviposition by females in different treatments during the days after adult emergence had the same pattern and is shown in figure 1. Maximum number of oviposited eggs was in the days 4th-9th after adult emergence in all treatments.

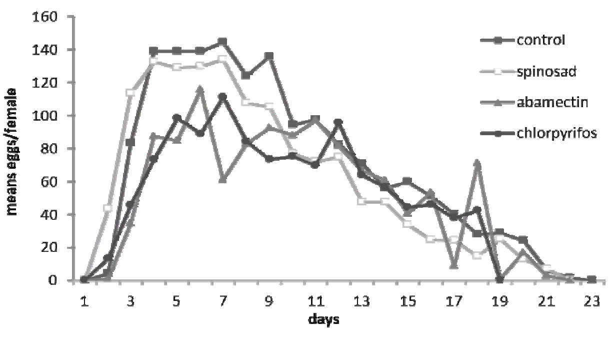

Figure 1. Means eggs produced by *H. armigera* that emerged from treated third instar larvae and control.

Discussion

Results obtained from acute toxicity assays of the tested insecticides showed that chlorpyrifos and spinosad were the most toxic chemicals against cotton bollworm. Similar results were found by Aslam *et al*. (2004), who have reported that chlorpyrifos was the most effective insecticide for controlling cotton bollworm among tested insecticides. Nirmal and Manjit (2008) reported that the LC_{50} values of spinosad and chlorpyrifos were 0.04 and 0.66 ppm, respectively. Differences in LC_{50} values of these two studies would be due to differences in the duration of exposure to the insecticide and larval instar. Therefore, according to our results and the mentioned studies spinosad and chlorpyrifos have high potential for controlling cotton bollworm.

Chlorpyrifos had the highest slope among the test insecticides (table 1). The high slope indicates that a slight increase in insecticide concentration will lead to high mortality compared with the other insecticides. However, the higher slope indicates that there will be increased selection pressure on the population. Thus, there will be a greater risk of selection of resistant individuals compared with the other insecticides especially in cases of continued use of the same insecticide.

The current study demonstrated that abamectin and chlorpyrifos significantly reduced the pupal weight, longevity and fecundity of adult cotton bollworm. Spinosad did not affect the pupal period, pupal weight and fecundity of the adults. Furthermore, spinosad had no effect on longevity in compared with control. The results of present study are in agreement with those of Nirmal and Manjit (2008) who reported that LC_{30} and LC_{50} values of chlorpyrifos significantly reduced the means of oviposition of cotton bollworm compared with control. Furthermore, they have reported that LC_{50} values of spinosad did not affect the longevity of cotton bollworm, but LC_{30} values of spinosad significantly reduced the oviposition compared with control. In contrast with our results, Wang *et al.* (2009) reported that 0.04 and 0.16 mg kg^{-1} of spinosad increased the pupal periods and reduced pupal weight. It also reduced longevity and fecundity of adult cotton bollworms. Sublethal dose of spinosad increased the fecundity of *Orius insidious* (Say) compared with control, but this increase was not significant (Elzen, 2001). Pineda *et al.* (2007) reported that spinosad reduced the fecundity and fertility of *Spodoptera littoralis* (Boisduval) adults when treated orally and residually. The type of formulation of the insecticides, differences among the populations used and exposure methods may account for the different results in our study and those of others.

In addition, it was demonstrated that chlorpyrifos and spinosad had high acute toxicity against cotton bollworm. Abamectin and clorpyrifos negatively affected the longevity and fecundity of the pest. These impacts are very important for practical management of the pest, because these effects may lead to the reduction of the pest population to a lower level even under economic injury level. Therefore, the authors propose that both lethal and sublethal effects of the insecticides should be considered in developing of a pest management program.

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