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Toxicity of emamectin benzoate and cypermethrin on biological parameters of cotton bollworm, *Helicoverpa armigera* (Hübner) in laboratory conditions

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Abstract: Cotton bollworm, *Helicoverpa armigera* (Hübner) is one of the most destructive insect pests on many crops in the world that has been found to develop resistance against conventional insecticides. Using insecticides with different modes of action may result in appropriate control of the pest and delay insecticide resistance development. In this study, lethal and sublethal effects of emamectin benzoate and cypermethrin insecticides were investigated on third instar larvae of *H. armigera* by residue contact methods at 26 ± 1 ^{o}C, 70 ± 5% RH and a photoperiod of 16:8h (L: D) under laboratory conditions. LC_{50} values, on larval stage of the pest, of emamectin benzoate and cypermethrin were 1.75 and 127.74 µg a.i./ml, respectively. According to the findings, the larvae that were exposed to the LC_{30} of emamectin benzoate and cypermethrin exhibited lower pupal weight and increased larval and pupal developmental times compared with control. The longevity and fecundity of adults were significantly affected by the insecticides. Emamectin benzoate and cypermethrin reduced fecundity by 53.1% and 50.5%, respectively compared to control. The LC_{30} values of emamectin benzoate and cypermethrin reduced egg hatching by 62.06% and 37.9%, respectively. It is predicted that these insecticides, especially emamectin benzoate, may induce significant effects on population of *H. armigera*.

Keywords: cotton bollworm, lethal and sublethal effects, longevity, fecundity

Introduction

Cotton and tomato are among the major economic crops in Iran and *H. armigera*, has been a major problem for cotton and tomato production. The cotton bollworm is highly polyphagous, multivoltine and economically important pest of cotton and other crops (Nair *et al*., 2010), and causes severe damage and loss to a wide range of food, fiber, oil, fodder, vegetable, horticultural, ornamental, aromatic and medicinal plants (Nadda *et al*., 2012). This pest has exhibited resistance to all of the conventional chemicals such as endosulfan, pyrethroids, organophosphates and carbamates (Ahmad *et al*., 2003). Potential non-pyrethroid alternatives are biorational insecticides (spynosins, oxadiazines, avermectins, etc.) which have no cross resistance with pyrethroids, are more specific, require lower rates, degrade more rapidly in the environment, and have lower mammalian and aquatic toxicity (Holloway *et al*., 1999). Therefore, new and environmentally friendly approaches are urgently needed for

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controlling this pest. Using new types of insecticides, originated from natural agents or products that disrupt the physiological processes of the target pest, could be useful as an alternative for the integrated management approach (Smagghe *et al*., 2003). Emamectin benzoate belongs to the avermectin group of chemicals produced by the soil-dwelling actinomycete (NRRL 8165) alias, *Streptomyces avermitilis* (Burg *et al*., 1979). Emamectin benzoate targets various lepidopteran pests and is being developed for use on major field crops and vegetables, such as soybean, cotton, cabbage and radish (Ishaaya *et al*., 2002). Cypermethrin is a synthetic pyrethroid insecticide that has high insecticidal activity, low avian and mammalian toxicity, and adequate stability in air and light. It is used to control many pests including lepidopterous pests of cotton, fruit, and vegetable crops (Jones, 1995). Sublethal effects may be manifested as reductions in life span, development rates, fecundity, changes in sex ratio, and changes in behavior (Croft, 1990; Stark and Banks, 2003). Therefore, the study of lethal and sublethal effects of insecticides with different modes of action such as emamectin benzoate and cypermethrin may show high toxicity against cotton bollworm and provide a suitable pest management program. The main objective of this study was to evaluate lethal and sublethal effects of emamectin benzoate and cypermethrin on *H. armigera* under laboratory conditions to be used in combination with biological control agents.

Materials and Methods

Insect culture

A laboratory colony of the cotton bollworm, *H. armigera* was originated from cotton fields of Moghan region in North West of Iran. Larvae of cotton bollworm were reared on artificial diet at laboratory conditions with 25 ± 1 ^{o}C, 70 ± 5% RH and a photoperiod of 16:8h (L: D) (Saber *et al*., 2012). The artificial diet included cowpea powder 205 g, methyl-p-hydroxybenzoate 2.2 g, ascorbic acid 3.5 g, wheat germ 30 g, powdered agar 14 g, sorbic acid 1.1 g, yeast 35 g, formaldehyde 37% 2.5 ml, vegetable oil 5 ml and distilled water 650 ml (Shorey and Hale, 1965). For preventing cannibalism, the third instars were transferred into individual glass vials (3 × 9 cm) and were maintained until pupation. After appearance, 20 pairs of adult moths were released into 20 × 30 cm plastic containers with 1:1 sex ratio for mating and egg-laying. The adults were fed on a 10% honey solution.

Insecticides

The tested insecticides were cypermethrin (Patron® 40% EC Aryashimi Co., Iran) and emamectin benzoate (Proclaim® 5% SG, Syngenta Co., Switzerland).

Bioassays

The toxicity of cypermethrin and emamectin benzoate was assessed on newly molted third instar larvae of the cotton bollworm. Third instar larvae were exposed to the tested insecticides by residue contact methods. The preliminary concentration-setting experiments were carried out to determine the main concentrations for bioassay test. The main concentrations were 335.6, 240, 165.2, 115.6, 80.8, 56.8, 40 and 28 µg a.i./ml for cypermethrin, 5, 3.15, 1.99, 1.25, 0.79 and 0.5 µg a.i./ml for emamectin benzoate. Each concentration involved 3 replications and each bioassay test was replicated three times. 3 ml of concentrations were applied on glass petri dishes by residue contact method. After drying petri dishes, 20 third instar larvae were transferred into glass petri dishes (9 cm in diameter by 3 cm in height) for each cypermethrin and emamectin benzoate concentration. Distilled water was used for control treatment. Then, the petri dishes were transferred to the growth chamber set at 26 ± 1 ^{o}C, 70 ± 5% RH and a photoperiod of 16:8h (L: D). Mortality was recorded after 24 h in all experiments, if no movement was observed larvae were considered dead.

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Sublethal effects

Sublethal effects associated with emamectin benzoate and cypermethrin were evaluated on third instar larvae of *H. armigera*. For experimentation, 100 third instar larvae of cotton bollworm were treated with LC_{30} of each insecticide by residue contact methods. After 24 hours, the survivors were kept in individual glass vials and fed on untreated artificial diet until pupation. The pupal weight and life span of larvae and pupae were recorded after pupation. The influence of insecticides on fecundity, longevity and hatching rate was assessed on pairing moths in a small mating chamber lined and covered with chiffon. To study the effect of treatments on hatching rate, 30 eggs each of emamectin benzoate, cypermethrin and control treatments were sampled and until hatching were kept at 26 ± 1 ^{o}C, 70 ± 5% RH and a photoperiod of 16: 8h (L: D). Four replications were used. The mating chambers were provided with 10% honey solution on a moist cotton trough that was replaced every day. The number of eggs laid by females was recorded daily until each female died.

Statistical analysis

To calculate LC_{50} and LC_{30} values, the data were analyzed using probit procedures with SAS program v. 9.2 (SAS Institute, 2002). Normalized test were done before statistical analysis and data were normal. Differences in the duration of the larval stage, pupal weight, duration of the pupal stage, fecundity, and longevity were analyzed by ANOVA with mean separation at a 5% level of significance by the LSD test using SAS (SAS Institute, 2002).

Results

Larval toxicity bioassay

The LC_{50} values indicated that emamectin benzoate was more toxic on *H. armigera* compared with cypermethrin (Table 1). The toxicity of insecticides tested was significantly different.

The effects of LC_{30} value of the insecticides on the larval and pupal stages of *H. armigera* are shown in Table 2. Both insecticides significantly affected the duration of the larval period ($F = 12.42$; $df = 2, 66$; $P < 0.0001$). Emamectin benzoate and cypermethrin prolonged the duration of the larval stage by 3.5 and 3.3 days, respectively, compared with control. The duration of the pupal stage ($F = 7.4$; $df = 2, 22$; $P = 0.003$) and pupal weight ($F = 14.7$; $df = 2, 73$; $P < 0.0001$) of insects exposed to LC_{30} of emamectin benzoate and cypermethrin were also significantly affected. Exposure to an LC_{30} of emamectin benzoate and cypermethrin increased the pupal period by 2.6 and 1.9 days, respectively (Table 2).

Sublethal effects on adults

LC_{30} values of the insecticides significantly affected male and female longevity of *H. armigera* in comparison to control insects (Male: $F = 6.6$; $df = 2, 5$; $P = 0.04$- Female: $F = 49.9$; $df = 2, 10$; $P < 0.0001$) (Table 3). The female longevity of adults exposed to an LC_{30} of emamectin benzoate or cypermethrin was reduced by 8.5, and 6.3 days, respectively. Also the male longevity of adults was reduced by 10.7 days for emamectin benzoate and 5.7 days for cypermethrin. Emamectin benzoate significantly decreased male and female longevity compared with control. In addition, exposure to LC_{30} values of both compounds significantly reduced the number of eggs laid in comparison with control insects during their life span ($F = 12.2$; $df = 2, 10$; $P = 0.002$) (Table 3). Specially percentage of hatched eggs of females survived after exposuring to emamectin benzoate and cypermethrin was 27.5% and 45%, respectively while this value was 72.5% for the control females ($F = 90.31$; $df = 2, 9$; $P < 0.0001$) (Table 3).

Oviposition of females in different treatments during the days after adult emergence had the same pattern and is shown in (Fig. 1). Maximum number of oviposited eggs was in the days 4^{th}-9^{th} after adult emergence in all treatments.

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Table 1 Toxicity of emamectin benzoate and cypermethrin on third instar larvae of *H. Armigera.*

Insecticide	R^2	Slope ± SE	χ^2	Lethal concentrations (µg a.i./ml)		
				LC_{30} (95%FL)	LC_{50} (95%FL)	LC_{90} (95%FL)
Emamectin benzoate	0.94	1.46 ± 0.22	42.69	0.77 (0.5-1.01)	1.75 (1.39-2.23)	13.08 (7.81-32.95)
cypermethrin	0.99	560 1.48 ± 0.22	44.81	56.5 (38.86-72.77)	127.74 (101.75-165.76)	939.20 (552.17-2400)

Sublethal effects on pre-imaginal stages

Table 2 Sublethal effects of emamectin benzoate and cypermethrin on larval and pupal stages of *H. armigera.*

Insecticides	Duration of larval stage ± SE (d)	Pupal weight ± SE (mg)	Duration of pupal stage ± SE (d)
Emamectin benzoate	23.1 ± 0.6 b	210.4 ± 4.3 b	12.3 ± 0.8 b
Cypermethrin	22.9 ± 0.7 b	220.6 ± 5.4 b	11.6 ± 0.4 b
Control	19.6 ± 0.7 a	248.1 ± 5.4 a	9.7 ± 0.4 a

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

Table 3 Sublethal effects of emamectin benzoate and cypermethrin on *H. armigera* adults.

Insecticide	Male longevity ± SE (day)	Female longevity ± SE (day)	Mean eggs per female ±SE	Egg hatchingrate (%) ± SE
Emamectin benzoate	6.5 ± 2.5 b	11.5 ± 0.6 c	456.0 ± 64.2 b	27.5 ± 2.1c
cypermethrin	11.5 ± 0.5 ab	13.7 ± 0.5 b	481.3 ± 72.2 b	45.0 ± 2.2 b
Control	18.5 ± 2.3 a	20.0 ± 0.7 a	973.6 ± 102.5 a	72.5 ± 2.8 a

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

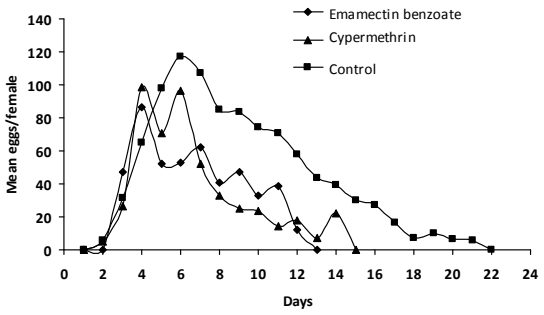

Figure 1 Mean eggs produced by *H. armigera,* emerged from treated third instar larvae with emamectin benzoate and, cypermethrin.

Discussion

The results of the current study showed that emamectin benzoate insecticide was highly toxic to *H. armigera*. Emamectin benzoate is highly effective against a broad spectrum of lepidopterans. LC_{90} values for emamectin benzoate against several lepidopterans pests such as *Manduca sexta* Linnaeus, *Plutella xylostella* Linnaeus, *Helicoverpa virescens* Fabricius, *Trichoplusia ni* Hübner, *Helicoverpa zea* Boddie, *Spodoptera exigua* Hübner, *Spodoptera eridania* Stoll, *Spodoptera frugiperda* J. E. Smith,

[DOR: 20.1001.1.22519041.2013.2.4.7.1]

Pseudoplusia includes Walker, *Ostrinia nubilalis* Hübner, *Agrotis ipsilon* Hufnagel, *Argyrotaenia velutinana* Walker, *Cydia pomonella* Linnaeus ranged between 0.002-0.89 µg/ml (Jansson and Dybas, 1998). Argentine *et al*., (2002) found that the LC_{90} values for emamectin benzoate ranged from 0.005 to 0.021 µg/ml for six species of Lepidoptera. We found that LC_{90} for emamectin benzoate against *H. armigera* was 13.08 µg a.i./ml. This discrepancy could be due to difference in tested population. Dunbar *et al*., (1998) reported that emamectin benzoate was very effective in controlling *Helicoverpa virescens* and *Helicoverpa zea* larvae. Furthermore, White *et al*., (1997) reported that emamectin benzoate provides ecological selectivity to a wide range of beneficial arthropods and is compatible with integrated pest management programs. In contrast cypermethrin had lower efficacy on 3rd instar *H. armigera*. The lower toxicity of cypermethrin to control *H. armigera* contrasts the findings of Ahmed *et al*., (2004). According to comparative study on *H. armigera* by Brevault *et al*., (2009), field rate of emamectin benzoate and cypermethrin caused 82.2% and 48.7% mortality in 3rd instar larvae of *H. armigera*, respectively. Sublethal effects such as reductions in reproductive capacity, longevity, and pupal weight of survivors will likely result in negative impacts on insect population dynamics (Trisyono and Chippendale, 1998; Knight, 2000; Pineda *et al*., 2007). In this study, we found that sublethal exposure to emamectin benzoate and cypermethrin increased the duration of larval and pupal stages. Our results are consistent with Lixia *et al*., (2011) that reported LC_{25} concentration of emamectin benzoate prolonged larval and pupal period. A similar increase in duration of larval and pupal stages was found in 3rd instar larvae of *H. armigera* that were exposed to LC_1 and LC_{50} values of beta-cypermethrin (Hui-xian *et al*., 2005). Effect of cypermethrin on larval growth in the tests is in agreement with those reported by Rimoldi *et al*., (2011) who found that the lowest cypermethrin concentration tested significantly inhibited larval growth with respect to the control.

The tested insecticides significantly reduced pupal weight. Reduction of pupal weight resulted by emamectin benzoate in our study contrasts with those reported by Lixia *et al*., (2011). The reason for this discrepancy requires further investigation. Hui-xian *et al*., (2005) detected decrease of pupal weight with sublethal concentration of beta cypermethrin and deltamethrin. These researchers indicated that pyrethroids at sublethal levels had negative effects on the biology of the cotton bollworm. According to the concepts considered within the IPM paradigm, studying the biological effects of pesticides on target organisms is very relevant for the minimization of treatment thresholds and the assessment of new pesticide efficiency. Moreover, the sublethal effects could also contribute to reduce pest population levels (Rimoldi *et al*., 2011). In the current study, it was found that exposure to sublethal concentration of two classes of insecticides induced significant effects on the reproductive capacity and adult longevity of *H. armigera*. In consistens with this, a significant reduction in fecundity was observed in *H. zea* that were exposed to a sublethal concentration of emamectin benzoate (Lopez *et al*., 2010). In contrast, Hari and Mahal (2011) stated that egg laying by female adults of *H. armigera* in the cotton bolls was higher in case of cypermethrin application at LC_{50} than in control. This discrepancy could be due to difference in testing methods and other factors such as larval age and experimental conditions. Specifically, we demonstrated that insecticides had negative effects on adult longevity of *H. armigera* under laboratory conditions. Emamectin benzoate caused considerable reduction in adult longevity. In the present study, cypermethrin significantly reduced female longevity of *H. armigera*,

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whereas, there was no significant reduction in male longevity compared with control insects. Several studies have reported reduced adult longevity by cypermethrin. For example, Ergin *et al*., (2007) examined effect of cypermethrin exposed hosts on egg to adult development time, number of offspring, sex ratio, longevity, and size of *Apanteles galleriae* Wilkinson (Hymenoptera: Braconidae). Likewise, reduction of adult longevity was observed by El-ghar and El-sayed (1992) to methomyl, tralomethrin and cypermethrin on *Diaeretiella rapae* McIntosh. In the present study, the lethal and sublethal effects of emamectin benzoate and cypermethrin were verified on *H. armigera* larvae. The results showed that emamectin benzoate negatively affected most of the life parameters that were investigated. From a practical point of view, the sublethal effects of emamectin benzoate are highly important because a reduction in the number of offspring will likely help to drive down the field insect population. Furthermore, the combination of lethal and sublethal effects of insecticides such as emamectin benzoate will likely produce negative effects on the population dynamics of *H. armigera*. Sublethal concentrations of insecticides may be applied in combination with agents of biological control for pest management due to low effects of these concentrations against natural enemies compared with field recommended concentrations. In comparison to cypermethrin, emamectin benzoate showed higher acute toxicity against the larval stages of *H. armigera*. On the basis of our findings, we conclude that emamectin benzoate has higher potential for controlling *H. armigera*. Final evaluation of the use of this chemical compound should be considered after field studies.

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سمیت امامکتین بنزوآت و سایپرمترین روی پارامترهای بیولوژیکی کرم قوزه پنبه *Helicoverpa armigera* (Hübner) تحت شرایط آزمایشگاهی

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چکیده: کرم قوزه پنبه*Helicoverpa armigera* Hübner یکی از آفات مهم در جهان است که به اکثر حشره‌کش‌های مرسوم مقاومت نشان داده است. استفاده از حشره‌کش‌های با نحوه اثر متفاوت ممکن است کنترل مناسبی در برابر این آفت فراهم کند و توسعه مقاومت به حشره‌کش‌ها را کاهش دهد. در این آزمایش اثرات کشندگی و زیرکشندگی امامکتین بنزوآت و سایپرمترین روی مرحله لاروی کرم قوزه پنبه مورد ارزیابی قرار گرفت. مقادیر LC_{50} امامکتین بنزوآت و سایپرمترین روی لاروهای این آفت به‌ترتیب برابر ۱/۷۵ و ۱۲۷/۷۴ میکروگرم ماده‌ی مؤثر/ میلی‌لیتر بود. با توجه به نتایج به‌دست آمده، لاروهای در معرض قرار گرفته با غلظت‌های LC_{30} امامکتین بنزوآت و سایپرمترین در مقایسه با لاروهای شاهد، وزن کمتری داشتند و باعث افزایش طول دوره شفیرگی گردیدند. طول عمر و باروری حشرات کامل به‌طور معنی‌داری تحت تأثیر حشره‌کش‌ها قرار گرفتند. غلظت‌های زیرکشنده امامکتین بنزوآت و سایپرمترین در مقایسه با شاهد باروری حشرات کامل را به‌ترتیب ۵۳/۱ و ۵۰/۵ درصد کاهش دادند. همچنین مقادیر LC_{30} امامکتین بنزوآت و سایپرمترین میانگین تفریخ تخم را در مقایسه با شاهد به‌ترتیب ۶۲/۰۶ و ۳۷/۹ درصد کاهش دادند. پیش‌بینی ما این است که این حشره‌کش‌ها به‌ویژه امامکتین بنزوآت اثرات معنی‌داری را روی جمعیت *H. armigera* ایجاد کند.

واژگان کلیدی: کرم قوزه پنبه، اثرات کشندگی و زیرکشندگی، طول عمر، باروری

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