Field Performance and Laboratory Toxicity of Five Insecticides Against Black Cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae)

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ABSTRACT

Field evaluation of four conventional insecticides and one microbial insecticide against black cutworm (BCW), *Agrotis ipsilon*, at two locations in cotton during 2008 and 2009 seasons were carried out in this study. Susceptibility of the two field strains compared to the laboratory strain for the five insecticides also was studied in the laboratory. At Abou-Elmatameer location, triazophos and *Bacillus thuringiensis var. kurstaki* revealed the highest reduction percentages of BCW all over the experiment period during the two seasons. Triazophos caused BCW reduction percentages 91.7, 93.2. 94.1 and 94.5% during 2008 season, and 88.4, 90.6, 90.9 and 89.9% during 2009 season, after 3-, 6-, 9- and 12-days post-treatment, respectively. *Bacillus thuringiensis kurstaki* reduced BCW population by 68.2, 90.0, 92.7 & 93.2% at 2008 and 69.7, 87.3, 90.6 & 90.5% at 2009 after 3-, 6-, 9- and 12-days post-treatment, respectively. On the other hand, λ-cyhalothrin gave the least BCW control. At Abees location, *B.t. kurstaki* recorded the highest BCW reduction percentages in the two seasons. During 2008, *B.t. kurstaki* achieved 73.7, 87.1, 90.0 and 89.4% reduction in BCW population after 3-, 6-, 9- and 12-days post-treatment. These reduction percentages were 71.7, 90.5, 89.1 and 90.9% after 3-, 6-, 9- and 12-days post-treatment, respectively, at 2009. Reduction percentages of BCW achieved by carbosulfan, chlorpyrifos, triazophos and λ-cyhalothrin at 2008 and 2009 were comparable and significantly lower than *B.t. kurstaki*. Black cutworm reduction percentages achieved by carbosulfan, chlorpyrifos, triazophos and λ-cyhalothrin at Abou-El-Matameer were significantly higher than at Abees in the two seasons. On the other hand, at the two seasons, BCW reduction percentages caused by *B.t. kurstaki* were not significantly different at the two locations. At the laboratory, Abees strain exerts tolerance ratios higher than Abou-Elmatameer strain to the tested conventional insecticides. The two field strains had no tolerance to *B.t. kurstaki* compared to the laboratory strain. Therefore, *B.t. kurstaki* may be considered as a good alternative for controlling BCW.

INTRODUCTION

The black cutworm (BCW), *Agrotis ipsilon* (Hufnagel), is found in many regions worldwide and feeds on a wide range of plants (Harrison and Lynn, 2008). In Egypt, it is an economic pest attacking field and vegetable seedlings, such as cotton, soybean, corn, potatoes and tomatoes (Abo El-Ghar *et al*., 1996; Abd El-Aziz *et al*., 2007). Once the fourth instar is attained, larvae cause considerable damage. A larva may cut several plants in a night. The injury caused by larvae and their habits necessitates special efforts to control the pest (Zaki, 1996).

In cotton BCW causes severe damage when planting cotton after legume cover crops (Oliver and Chapin, 1981). In this case legumes serve as oviposition sites before planting, and larvae then move onto emergent cotton plants (Gaylor *et al*., 1984). Control of the BCW has depended exclusively on insecticides. As a result, this pest may develop resistance to those insecticides in many areas. Therefore, the continuous evaluation of the insecticides efficiency for controlling the insect in different areas became urgent. This will give the chance to replace the failed controlling agents by the effective alternatives. Moreover, the establishment of baseline susceptibility and the mechanism of resistance are necessary for effective resistance management strategies (Regupathy, 1996; Denholm *et al*., 1999; Mohan and Gujar, 2003).

The present study reports the effectiveness of certain insecticides in controlling the BCW in two areas. This study was conducted also to investigate the variation in susceptibility of two field populations of BCW to certain selected insecticides compared to the laboratory strain.

MATERIALS AND METHODS

Field studies: Field trials were conducted during two cotton seasons 2008 and 2009 at two different cities, Abou-Elmatameer at El-Behira Governorate and Abees at Alexandria Governorate. Cotton variety Giza 70 was cultivated at April 3 and 5 during 2008 season and April 10 and 11, during 2009 season, for the Abou-Elmatameer and Abees experiments, respectively. All cultural practices were carried out according to “good agricultural practice”. All treatments in addition to control were assigned to plots in a randomized complete block design with four replicates (each was 100 m^2 in area).

Preparation of baits: Baits were prepared as described by Salama *et al.*, (1995). For each treatment, each insecticide at the recommended field rate (Table 1) was

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Table 1. Evaluated insecticides and their application rates

Trade name	Common name	Rate / Fed.
Marshal 25%WP	carbosulfan	1.00 kg
Dursban 48%EC	chlorpyrifos	1.00 liter
Hostathion 40%EC	triazophos	1.25 liter
Dipel 2X 6.4%WP	*Bacillus thuringiensis kurstaki*	0.35 gm
Katron 5%EC	λ-cyhalothrin	1.00 liter

added to 100 ml water. Each insecticide solution was added to bran (2.5 kg), and molasses (0.4 kg) were then added and mixed well. Water was added gradually by suitable volumes (approx. 1 liter) as the baits required. Baits were prepared just before field application and the ingredients were completely mixed to ensure homogenous distribution of the ingredientsSowing date were on 3 and 5 May in the two seasons, respectively.

Field application of baits: The baits were distributed manually on the soil surface behind each hill. Application dates were May 5 and 6, for the Abou-Elmatameer and Abees experiments, respectively, during 2008 season and May 10 and 11, for the Abou-Elmatameer and Abees experiments, respectively, during 2009 season.

Estimation of infestation by BCW larvae: The pre-treatment counts of BCW larvae / 25 plants in each replicate (100 plants per each treatment) were made one day before the treatment. The numbers of BCW larvae / 25 plants in each replicate were counted 3, 6, 9 and 12 days post-treatment. Reduction percentages in the BCW larvae were calculated according to the formula of Henderson and Tilton (1955). The treatments were compared with each other using one way ANOVA with $LSD_{0.05}$ (CoStat Statistical Software, 1990).

Laboratory bioassay: Laboratory strain of the BCW was obtained from Plant Protection Research Institute and reared on castor oil plant leaves away from any insecticidal contamination under controlled laboratory conditions at 27 ± 2°C and 65 ± 5 % R.H. Larvae of field strains (Abou-El-Matameer and Abees) were collected during 2009 season from untreated areas, transferred to the laboratory and reared as described above for one generation to be used for the laboratory bioassay. Toxicity of the insecticides (Table 1) against the 2^{nd} instar larvae of BCW was evaluated. Homogenous pieces of the castor oil leaves were dipped in a series of each insecticide concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated castor oil leaf pieces were transferred to a plastic cups, and 10 starved larvae were added. Each concentration was replicated five times. Mortality percentages were recorded after 24 hr of treatment for the synthetic insecticides and after 4 days for *B. t*. Mortality percentages were corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971).

RESULTS

Field experiments:

Reduction percentages of BCW population after different time intervals of insecticides application at Abou-Elmatameer region are presented in Tables (2 and 3). All insecticides except *B.t. kurstaki* had high reduction percentages after 3-days of insecticides application. All over the experiment period, triazophos and *B.t. kurstaki* revealed the highest reduction percentages of BCW. Triazophos caused BCW reduction percentages 91.7, 93.2. 94.1 and 94.5% after 3-, 6-, 9- and 12-days post-treatment, respectively, during 2008 season. These reduction percentages were 88.4, 90.6, 90.9 and 89.9% after the same days post-treatment, during 2009 season. *Bacillus thuringiensis kurstaki* reduced BCW population by 68.2, 90.0, 92.7 & 93.2% at 2008 and 69.7, 87.3, 90.6 & 90.5% at 2009 after 3-, 6-, 9- and 12-days post-treatment, respectively. On the other hand, λ-cyhalothrin gave the lowest reduction percentages 75.5, 78.4, 79.1 & 78.3% at 2008 and 74.0, 75.6, 74.2 & 72.7% at 2009 after 3-, 6-, 9- and 12-days post-treatment, respectively (Tables 2 and 3).

Data in Tables (4 and 5) show the reduction percentages in BCW population due to the tested insecticides in the Abees experiments (2008 and 2009 seasons). It is clear that, the highest BCW reduction percentages in the two seasons were recorded by *B.t. kurstaki*. During 2008 season, *B.t. kurstaki* achieved 73.7, 87.1, 90.0 and 89.4% reduction in BCW population after 3-, 6-, 9- and 12-days post-treatment (Table 4). Reduction percentages were 71.7, 90.5, 89.1 and 90.9% after 3-, 6-, 9- and 12-days post-treatment, respectively, at 2009season (Table 5). Reduction percentages of BCW achieved by carbosulfan, chlorpyrifos, triazophos and λ-cyhalothrin at 2008 and 2009 were comparable and significantly lower than *B.t. kurstaki*.

Comparison between the field performances of each tested insecticide at Abou-El-Matameer and Abees experiments in the two seasons are presented in Figure (1). It is obvious that, BCW reduction percentages achieved by carbosulfan, chlorpyrifos and triazophos at Abou-Elmatameer were significantly higher than at Abees at the two seasons. On the other hand, in the two seasons, BCW reduction percentages caused by *B.t. kurstaki* and λ-cyhalothrin were not significantly different at the two locations.

Toxicity of tested insecticides against the 2nd instar larvae of BCW in the laboratory:

Susceptibility of field strains of BCW compared to the laboratory strain for carbosulfan, chlorpyrifos, triazophos, λ-cyhalothrin and *B.t. kurstaki* is given in Table (6). Generally, Abees strain exerts tolerance ratios higher than Abou-Elmatameer strain to the tested conventional insecticides. Abou-Elmatameer strain showed tolerance ratios of 3.68, 1.89, 1.83 and 1.34 to carbosulfan, chlorpyrifos, triazophos and λ-cyhalothrin, respectively. On the other hand, Abees strain showed tolerance ratios of 7.15, 8.67, 6.19 and 2.36 to the same insecticides, respectively. The two field strains didn't show any tolerance to *B.t. kurstaki* compared to the laboratory strain.

Table 2. Black cutworm reduction percentages after different time intervals of insecticides application at Abou-Elmatameer during 2008 season

Treatments	%Reduction			
	3-days	6-days	9-days	12-days
Carbosulfan	84.6 b	84.9 b	83.1 b	83.7 b
Chlorpyrifos	82.1 b	83.5 b	84.3 b	83.1 b
Triazophos	91.7 a	93.2 a	94.1 a	94.5 a
λ-cyhalothrin	75.5 c	78.4 c	79.1 c	78.3 c
B.t. kurstaki	68.2 d	90.0 a	92.7 a	93.2 a

Within the same column, numbers followed by the same letters are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05).

Table 3. Black cutworm reduction percentages after different time intervals of insecticides application at Abou-Elmatameer during 2009 season

Treatments	%Reduction			
	3-days	6-days	9-days	12-days
Carbosulfan	78.5 b	80.8 cd	79.7 cd	79.4 b
Chlorpyrifos	80.1 b	82.5 bc	83.1 bc	82.0 b
Triazophos	88.4 a	90.6 a	90.9 a	89.9 a
λ-cyhalothrin	74.0 c	75.6 d	74.2 d	72.7 c
B.t. kurstaki	69.7 d	87.3 a	90.6 a	90.5 a

Within the same column, numbers followed by the same letters are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05).

Table 4. Black cutworm reduction percentages after different time intervals of insecticides application at Abees during 2008 season

Treatments	%Reduction			
	3-days	6-days	9-days	12-days
Carbosulfan	70.3 a	73.5 b	76.2 b	75.7 b
Chlorpyrifos	68.1 a	70.6 b	71.4 b	73.2 b
Triazophos	73.4 a	75.2 b	75.7 b	76.0 b
λ-cyhalothrin	74.0 a	76.2 b	75.3 b	76.4 b
B.t. kurstaki	73.7 a	87.1 a	90.0 a	89.4 a

Within the same column, numbers followed by the same letters are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05).

Table 5. Black cutworm reduction percentages after different time intervals of insecticides application at Abees during 2009 season

Treatments	%Reduction			
	3-days	6-days	9-days	12-days
Carbosulfan	69.4 a	72.1 b	73.2 b	71.3 b
Chlorpyrifos	73.9 a	74.5 b	74.3 b	73.7 b
Triazophos	74.9 a	76.1 b	76.3 b	77.1 b
λ-cyhalothrin	72.0 a	73.6 b	74.7 b	73.8 b
B.t. kurstaki	71.7 a	90.5 a	89.1 a	90.9 a

Within the same column, numbers followed by the same letters are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05).

Table 6. Median lethal concentrations, confidence limits (CL) and slope ± standard errors of five selected insecticides against the 2nd instar larvae of black cutworm

Insecticides	Insect strain	LC_{50} (ppm)	95%CL	Slope ± SE	TR*
Carbosulfan	**Lab.**	6.8	5.1 – 9.4	1.8 ± 0.24	1.0
	Abou-Elmatameer	25.0	19.5 – 31.8	1.9 ± 0.30	3.68
	Abees	48.6	36.3 – 61.4	1.8 ± 0.27	7.15
Chlorpyrifos	**Lab.**	5.7	3.8 – 6.8	2.4 ± 0.37	1.0
	Abou-Elmatameer	10.8	7.4 – 15.4	1.7 ± 0.31	1.89
	Abees	49.4	36.1 – 64.3	1.9 ± 0.29	8.67
Triazophos	**Lab.**	3.6	1.6 – 5.7	2.4 ± 0.44	1.0
	Abou-Elmatameer	6.6	4.0 – 9.3	2.5 ± 0.37	1.83
	Abees	22.3	13.9 – 35.8	2.1 ± 0.23	6.19
λ-cyhalothrin	**Lab.**	4.7	3.8 – 6.0	2.1 ± 0.33	1.0
	Abou-Elmatameer	6.3	5.2 – 7.6	1.9 ± 0.24	1.34
	Abees	11.1	8.4 – 15.2	2.2 ± 0.37	2.36
B.t. kurstaki	**Lab.**	12.6	8.2 – 18.1	1.6 ± 0.18	1.0
	Abou-Elmatameer	14.8	9.6 – 20.0	1.7 ± 0.12	1.17
	Abees	15.8	10.1 – 24.8	1.8 ± 0.20	1.25

*TR = Tolerance ratio (LC_{50} of field strain / LC_{50} of laboratory strain).

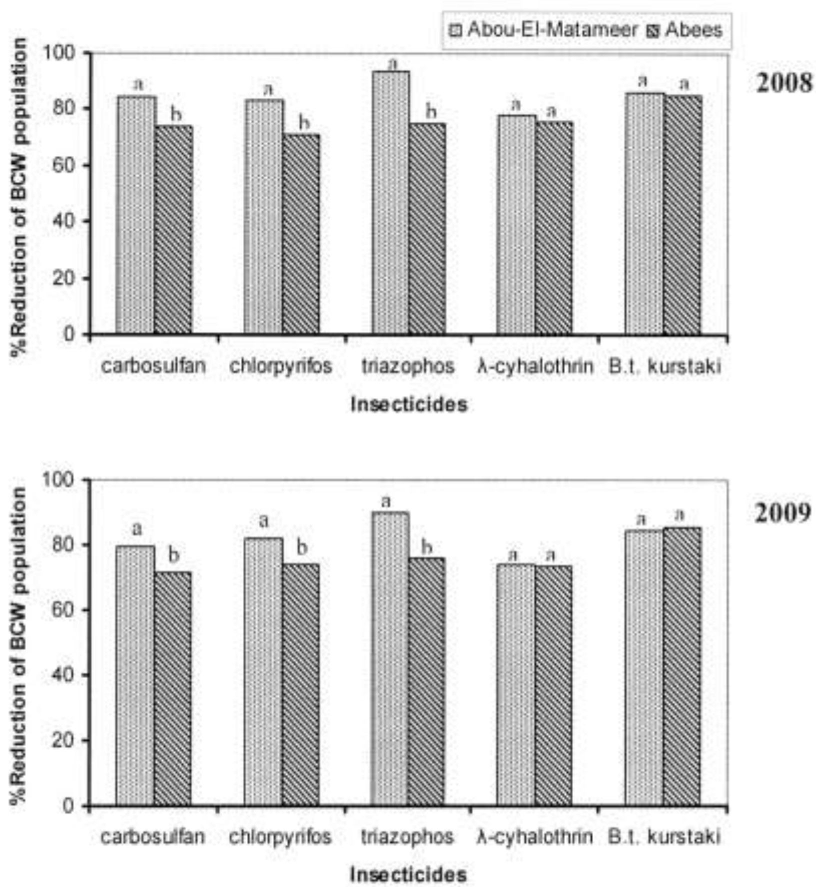

Figure 1. Comparison between reduction percentages of black cutworm achieved by the tested insecticides at Abou-El-Matameer and Abees at 2008 and 2009 seasons. Columns within a group with a letter in common are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05)

DISCUSSION

The black cutworm is one of the most severe insect pests in Egypt. The growers usually use the conventional insecticides, especially organophosphorus compounds, in controlling this pest. However, the intensive use of pesticides has resulted in environmental contamination (Frank *et al*., 1990; Abd El-Aziz *et al*., 2007). In this study, field performance of four synthetic insecticides and one bioinsecticide against BCW was evaluated. The bioinsecticide (depending on the *B.t. kurstaki*) is considered as a possible substitute and as a mean of reducing the use of chemicals in the environment. Results revealed that, all over the experiment period, triazophos and *B.t. kurstaki* revealed the highest reduction percentages of BCW. These results are in agreement with the results which reported by Salama *et al*., (1995). They found that, Dipel 2X® (*B.t. kurstaki*) and Hostathion® (triazophos) baits revealed a considerable BCW control in soybean fields. They concluded that, the use of biocontrol agent Dipel 2X® to control the BCW is of great value to soybean cultivations to avoid the residues of chemical insecticides on the seeds used to produce oil. In addition, the use of *B.t. kurstaki* was less harmful to the natural predators and parasites. Among the tested insecticides, λ-cyhalothrin, which gave the lowest BCW control. In respect of the λ-cyhalothrin, our results are at variance with those obtained by Mohamed, (2009). He reported that Karate® (λ-cyhalothrin) was significantly effective and gave the superior results in controlling BCW in commercial potato production fields.

The initial BCW reduction percentages (3-days after treatment) revealed by *B.t. kurstaki* at Abou-Elmatameer was low compared to the tested conventional insecticides. This may refer to the mode of action of *B.t* (need a time) which include 1) ingestion of sporulated *B.t* and insecticidal crystal protein (ICP) by an insect larva; 2) solubilization of the crystalline ICP in the midgut; 3) activation of the ICP by proteases; 4) binding of the activated ICP to specific receptors in the midgut cell membrane; 5) insertion of the toxin in the cell membrane and formation of pores and channels in the gut cell membrane, followed by destruction of the epithelial cells (Fast, 1981; Lüthy & Ebersold, 1981; Smedley & Ellar, 1996).

According to the laboratory bioassay, Abou-Elmatameer strain showed low tolerance ratios, while Abees strain showed higher tolerance ratios to the tested conventional insecticides. The two field strains had no tolerance to *B.t. kurstaki* compared to the laboratory strain. These results are in accordance with the field performance of these insecticides. Our results indicated to BCW reduction percentages achieved by carbosulfan, chlorpyrifos and triazophos at Abou-Elmatameer were significantly higher than at Abees at the two seasons. On the other hand, at the two seasons, BCW reduction percentages achieved by *B.t. kurstaki* were not different at the two locations. Therefore, *B.t. kurstaki* may serve as a good alternative to these insecticides in controlling the BCW in cotton fields.

Finally, further studies are needed to establish why BCW is showing the tolerance to the carbamate insecticide (carbosulfan) and the two organophosphorus insecticides (chlorpyrifos and triazophos) at Abees location. Also, further studies are needed to show the mechanisms of this tolerance.

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الملخص العربي

الكفاءة الحقلية والسمية المعملية لبعض المبيدات ضد الدودة القارضة *Agrotis ipsilon* (Lepidoptera: Noctuidae)

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فى هذه الدراسة تم التقييم الحقلى لأربعة مبيدات تقليدية وأحد المبيدات الميكروبية ضد الدودة القارضة فى موقعين مختلفين فى موسمى 2008، 2009. أيضا تم مقارنة حساسية السلالتين الحقليتين للخمسة مبيدات بالمقارنة بالسلالة المعملية فى المعمل.

أظهرت النتائج أن مبيدى الترايأزوفوس و الباسيلس ثيورينجينزز كيورستاكى كانا أكثر المبيدات المستخدمة خفضا ليرقات الدودة القارضة فى منطقة أبوالمطامير فى كلا موسمى الإختبار (2008، 2009). من ناحية أخرى أحدث مبيد لمبدا سيهالوثرين أقل نسبة خفض ليرقات الحشرة فى أبوالمطامير. فى منطقة أبيس أعطت الباسيلس ثيورينجينزز كيورستاكى أعلى خفض لتعداد يرقات الدودة القارضة فى كلا الموسمين.

عند مقارنة فاعلية المبيدات المستخدمة على الدودة القارضة فى كلا الموقعين وجد أن مبيدات الكربوسلفان، الكلوربيريفوس، الترايأزوفوس أعطت نسبة خفض مرتفعة معنويا فى منطقة أبوالمطامير مقارنة بمنطقة أبيس فى كلا موسمى التجارب. وفى نفس الوقت لم يكن هناك فرقا معنويا بين الموقعين بالنسبة لفاعلية الباسيلس ثيورينجينزز كيورستاكى. بالنسبة للإختبارات المعملية أظهرت سلالة أبيس درجة تحمل مرتفعة للمبيدات المختبرة عن سلالة أبوالمطامير وذلك عند مقارنتها بالسلالة المعملية ما عدا لمبيد الباسيلس ثيورينجينزز كيورستاكى الذى لم تظهر الحشرة له أى تحمل فى كلا السلالتين مقارنة بالسلالة المعملية. لذلك يمكن إعتبار الباسيلس ثيورينجينزز كيورستاكى وسيلة مكافحة بديلة للمبيدات التقليدية للدودة القارضة.