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Efficacy of Some Foliar Fertilizers and Alternative Chemicals on the Spiny Bollworm, *Earias insulana* (Boisd.) Larvae (Lepidoptera: Noctuidae) and their Side Effect on Protease Activity

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ABSTRACT

The present study was carried out on cotton variety Giza 70 during two consecutive seasons of 2009 and 2010 in an Experimental Farm at Alexandria, Egypt. The study was conducted to evaluate two foliar nutrients, a plant growth promoter (ascobin) and two alternative chemical compounds compared with a standard insecticide (chlorpyrifos) against the spiny bollworm, *Earias insulana* (Boisd.) and to determine the possible incorporation of such compounds within a program of Integrated Pest Management (IPM) of the spiny bollworm. The effect of the tested compounds on larval Protease activity and on cotton yield was also investigated. The obtained results indicated that the highest efficacy was gained from the following foliar treatments Easterna Aminofert Plus[®] (EAP)/Greenzit S.P₁₀₀/Chlorpyrifos, EAP/Radiant[®]/Methoxyfenozide[®] and Radiant[®]/Methoxyfenozide[®]/Chlorpyrifos since they decreased the incidence of *E. insulana* infestation during the cotton season of 2009. The foliar treatments of Radiant[®]/Methoxyfenozide[®]/Chlorpyrifos and EAP/Radiant[®]/Methoxyfenozide[®] were also high efficient in reducing the level of the spiny bollworm infestation in the season of 2010. The application of EAP followed by Radiant[®] and Methoxyfenozide[®] in respect increased the cotton yield compared to the untreated control in both seasons of 2009 and 2010. The foliar treatments applied as a sequence of EAP/Greenzit[®] S.P₁₀₀/Radiant[®] and the sequence of Radiant[®]/Methoxyfenozide[®]/Chlorpyrifos increased cotton yield in seasons of 2009 and 2010, respectively. The consequent treatments of EAP/Radiant[®]/Methoxyfenozide[®] gave the highest Protease enzyme activity of *Earias insulana* larvae while that of Radiant[®]/Methoxyfenozide[®]/Chlorpyrifos showed the lowest activity.

Key words: Foliar fertilizers, alternative chemicals, protease

INTRODUCTION

The genus *Earias* occurs in Africa and two species *E. insulana* (Boised) and *E. biplaga* WIK have been identified. Both species are being commonly known as the spiny bollworm, the name being derived from the characteristic bristles or spines which are formed on the larva. *E. insulana* is a very important pest in Egypt and is therefore, also known as the Egyptian bollworm (Ripper and George, 1965).

The mitral damage to the cotton crop by the spiny bollworm occurs in the early stages of plant growth. As the cotton plant grows and produces flower buds and unripe fruit, the attack of the

spiny bollworm is diverted to those. In Egypt insecticides are being used for controlling bollworms. Insecticides can provide economical protection by killing insect pests that otherwise would cause significant loss. Nevertheless, the extensive use of insecticides in cotton field has seriously affected the population densities of the natural enemies (Yang *et al.*, 2002; Younis *et al.*, 2007). Moreover, the widespread application of pesticides might accelerate some cotton pests, mostly insects and mites to develop resistance to certain pesticides (Elzen and Hardee, 2003).

Fertilization or/and foliar nutrients sprays might be good tool to produce a profitable cotton crop that competes with weeds and able to out-grow and overcome the possible occurrence and problems resulted from diseases and insects damage. Foliar spray can also correct deficiencies of certain required nutrients in large amounts (macro-elements) and/or required in trace amounts (micro-elements) (Treshow, 1970; El-Naggar, 1998; Mesbah *et al.*, 2000; El-Naggar, 2009).

Therefore, the present study was adopted to evaluate certain treatments of foliar nutrients, a plant growth promoter (Ascobin®), a bio-insecticide (Radiant®) (spinetoram), an insect growth inhibitor (methoxyfenozide or Runner®) and an organophosphorous insecticide (Chlorpyrifos) in different sequences to minimize *E. insulana* injury to and to study their probable side effect on larval protease enzyme activity and cotton yield.

MATERIALS AND METHODS

Field experiments were carried out at the Experimental Farm of Regional Research Station, Plant Protection Research Institute Agriculture Research Centre, Alexandria governorate throughout two successive cotton growing seasons of 2009 and 2010. Whereas, in both seasons an area of half feddan was cultivated with cotton variety "Giza 88" on April 30th. The experimental design was the split one with three replicates as well as untreated check.

The experimental areas were divided into plots (0.01 feddan) (42 m²). Each plot was separated from the adjacent one by a half-meter belt to minimize the interference of spray drift from one treatment to the other. In the growing cotton seasons of 2009 and 2010, prior to sewing, the cotton seeds were dressed with the bio-fertilizer Microbin®. The divided experimental area sprayed with 6 foliar treatments in sequences beside the untreated control. The sequences included two foliar nutrients, plant growth promoter (Ascobin®), a bio-insecticide (Radiant®), an insect growth inhibitor (methoxyfenozide) and Chlorpyrifos as standard insecticide and these sequences and the rate of application of their components are shown in Table 1.

The consequent applications of each tested compound were performed at different periods, i.e., at the beginning of flowering stage, after the fifty percentage of flowering and at the beginning of fruiting stage. During the whole period of the growing season, inspections were carried out for determining the efficacy of the tested compounds on the infestation level of *E. insulana*, cotton yield and the larval Protease activity. Sprayings were performed using a knapsack sprayer (20 L).

Estimation of the spiny bollworm infestation: Weekly inspections were done to determine the infestation levels of the spiny bollworm. Samples of 10 green bolls/plots were taken randomly; examined bolls for each treatment were 30 green bolls represent three replicates. The weekly estimation of the bollworm infestation level was performed over ten weeks during both seasons. In each sample, bolls were examined externally before dissection and then internally. Infestation levels were based on the existence of injury symptoms regardless of the presence of the larvae.

Cotton yield: In each treatment, ripened open bolls from thirty cotton plants were collected to determine the rate of cotton yield/plant, from which, the total yield/feddan was relatively calculated.

Effect on protease enzyme activity: Alive larvae of the spiny bollworm, *E. insulana* of almost same age and weight were collected from each plot to determine protease activity. The method of determination is described by Charney and Tomarelli (1947).

TESTED COMPOUNDS

Foliar fertilizers: A-Easterna Aminofert Plus® (Liquid)(EAP) is produced by Easterna Company for Agriculture Development (Easterna, Egypt). The main components are: amino acids (10%), saccharides and (6%), nitrogen (6%), phosphorous (4%), potassium (6%), sulphur (5%), organic matter (85%), magnesium (5000 ppm), chelated ferric (1000 ppm), chelated zinc (500 ppm), chelated manganese (250 ppm), chelated copper (150 ppm) and boron (100 ppm).

B-Greenzit SP₁₀₀® is produced by Ciba Geigy limited (Basel, Swiss) and the main components are: EDTA Na₂Mn (40%) and EDTA Na₂Zn (43%) supplemented by Ca: 0.054, Fe: 5.40, Mo: 0.027, Mg: 0.54, Ni: 0.005, Mn: 5.54, Cu: 0.005 and Zn: 70.27 g kg⁻¹.

Plant growth promoter: Ascobin® is produced by General Organization Agricultural Economic Fund (G.O.A.E.F) (Egyptian Ministry of Agric.). The main components are ascorbic acid+citric acid (38 %) and organic matters (62 %).

The agrochemical pesticides A-spinetoram (Radiant® 12 SC): It is a semi synthetic compound is the second generation of spinosyns group. It is a trademark of Dow Agro Sciences Co. (Dow, England).

Insect growth inhibitor (IGI): Methoxyfenozide or Runner® 24% (SC); N-tert-butyl-N-(3,5- dimethylbenzoyl)-3-methoxy-2-methyl benzohydrazide. It was supplied by Rohm and Hass Co. (Dow, England).

Organophosphorous compound: Chlorpyrifos was used as Dursban® 48% (EC); O, O diethyl O-3, 5, 6 trichloro-2-pyridyl phosphorothioate (Dow, England).

The tested foliar as applied in different sequences (fine) compared with Chlorpyrifos as a standard insecticide and their rates of application are presented in Table 1. Data were subjected to the analysis of variance test (ANOVA), with mean separation at 5% levels of significance, Computer program COSTAT and Duncan's Multiple Rang Test was used to compare the averages according to the method of Snedecor and Cochran (1967).

Table 1: The tested foliar and their rates of application during the growing seasons of 2009 and 2010

Treatments (applied as sequenced sprays)	Rate of application L ⁻¹
EAP/Greenzit® SP ₁₀₀ /Ascobin®	10 mL/0.3 g/1 g
EAP/Greenzit® SP ₁₀₀ /Radiant®	10 mL/0.3 g/0.4 mL
EAP/Greenzit®SP ₁₀₀ /Chlorpyrifos	10 mL/0.3 g/5 mL
EAP/Radiant®/methoxyfenozide	10 mL/0.4 mL/0.5 mL
Radiant®/Methoxyfenozide/Chlorpyrifos	0.4 mL/0.5 mL/5 mL
Chlorpyrifos (standard)	5 mL

EAP: Easterna Aminofert Plus®

RESULT AND DISCUSSION

Estimation of the spiny bollworm infestation

Efficacy of the applied foliar treatments on the spiny bollworm infestation during season of 2009: As illustrated results in Table 2 the level of the spiny bollworm infestation is expressed as the mean numbers of detected spiny bollworm larvae/10 bolls. The tested foliar treatments sequences (EAP/Greenzit S.P₁₀₀/Chlorpyrifos, EAP/Radiant/Methoxyfenozide® and Radiant/methoxyfenozide®/Chlorpyrifos) decreased efficiently the spiny bollworm infestation to 0.44, 0.40 and 0.44 larvae/10 bolls, respectively while, the other tested foliar sequences, resulted in a higher infestation were obtained ranged from 0.62-0.77 larvae/10 bolls when it compared with the untreated check (1.81 larvae/10 bolls).

Efficacy of the applied foliar treatments on the spiny bollworm infestation during season of 2010: From data in Table 2, it could be noticed that the treatment of Radiant/methoxyfenozide®/Chlorpyrifos was highly efficient and gave the lowest level of spiny bollworm infestation not exceeding 0.48 larvae/10 bolls, when it compared with the untreated check (2.74 larvae/10 bolls), This was followed by the sequence of EAP/Radiant/Methoxyfenozide® (0.92 larvae/10 bolls). Although, the other applied foliar treatments gave somewhat higher infestation levels by spiny bollworm but it was less than the untreated check and ranged from 1.40 to 1.96 larvae/10 bolls.

Obtained results of the present work are in agreement with Amin and Gergis (2006), who found that the treatments of Agerin, *Trichogramma*, Cascade, Consult, Mimic, Spinosad and conventional insecticides reduced the infestation of the three tested pests (leaf worm , spiny bollworm and pink bollworm). Abdel-Rhman (2004) found that the highest reduction in the cotton bollworm infestation were induced by the use of Chlorpyrifos, Tracer® (Spinosad) and Methoxyfenozide®. Moreover, El-Naggar (2003) and Mesbah *et al.* (2004) illustrated that the application of Baythroid® with Greenzit S.P₁₀₀ have also lowered spiny bollworms infestation. Also, the obtained results by Purohit and Deshpande (1994), Mesbah *et al.* (2000) and El-Naggar (2009) were in full agreements with the present aforementioned and illustrated findings.

Table 2: The mean numbers of spiny bollworm larvae/10 bolls as affected by the tested foliar treatments throughout the growing seasons of 2009 and 2010

Treatments	Mean No. of larvae/10 bolls	
	2009	2010
EAP/Greenzit® SP ₁₀₀ /Ascobin®	0.77 ^b	1.77 ^{bc}
EAP/Greenzit® SP ₁₀₀ /Radiant®	0.66 ^{bc}	1.40 ^c
EAP/Greenzit®SP ₁₀₀ /Chlorpyrifos	0.44 ^f	1.44 ^f
EAP/Radiant®/Methoxyfenozide	0.40 ^c	0.92 ^d
Radiant®/Methoxyfenozide/Chlorpyrifos	0.44 ^f	0.48 ^e
Chlorpyrifos	0.62 ^{bc}	1.96 ^b
Untreated check	1.81 ^a	2.74 ^a
F-calculated	29.43	2.19
F-tabulated	14.40	2.19
LSD _{0.05}	0.254	0.36

Table 3: Effect of the tested foliar treatments on the cotton yields (Kent./feddan) in the growing seasons of 2009 and 2010

Treatments	2009		2010	
	Kent.*	Increase (%)**	Kent.*	Increase(%)**
EAP/Greenzit® SP ₁₀₀ /Ascobin®	4.72	82.35	3.80	150
EAP/Greenzit® SP ₁₀₀ /Radiant®	9.44	264.70	8.38	450
EAP/Greenzit®SP ₁₀₀ /Chlorpyrifos	4.72	82.35	2.28	50
EAP/Radiant®/methoxyfenozide	9.75	276.47	15.23	900
Radiant®/ Methoxyfenozide/Chlorpyrifos	8.38	223.52	13.71	800
Chlorpyrifos	7.61	194.11	3.04	100
Untreated check	2.59		1.52	

*A kentar (kent.) of seed cotton = 157.5 kg, **Expressed as % of increase than the untreated check, according to Hussin *et al.* (2002)

Effects on cotton yield

Efficacy of the applied foliar treatments on cotton yield during season of 2009: The exhibited results in Table 3 indicated that the application of EAP/Radiant®/methoxyfenozide® and EAP./Greenzit® S.P₁₀₀/Radiant® gave the highest cotton yield increase than untreated check (276.47 and 264.70%, respectively) followed by the sequence of Radiant®/methoxyfenozide®/Chlorpyrifos (223.52 %). On the other hand, the other tested foliar sequences gave increase in the cotton yield ranged from 82.35% in case of EAP/Greenzit® S.P₁₀₀/Ascobin® and EAP./Greenzit® S.P₁₀₀/chlorpyrifos to 194.11% in case of Chlorpyrifos.

Efficacy of the applied foliar treatments on cotton yield during season of 2010: As showed in Table 3 the performance of EAP/Radiant®/Methoxyfenozide® and Radiant®/Methoxyfenozide®/Chlorpyrifos registered the cotton yield increase (900 and 800 %) followed by the treatment of EAP/Greenzit® S.P₁₀₀/Radiant® (450%). Versus, the applied foliar sequences of EAP/Greenzit® S.P₁₀₀/Chlorpyrifos gave the lowest increase (50%). For the other applied foliar treatments, the estimated increase of cotton yield was more or less extent, lowered but still higher than the untreated control and amounted to 100 and 150% for Chlorpyrifos and EAP/Greenzit® S.P₁₀₀/Ascobin®, respectively.

The present results are in agreement with those of El-Naggar (2003) and Mesbah *et al.* (2004), who concluded that the application of bi-and/or tri-sequent sprays of the Baythroid® with Greenzit S.P₁₀₀, Greenzit N.P.K and Polymex/Ascorbic acid increased the yield of treated cotton plants. Abdel-Rhman (2004) stated that the treatments of Tracer® (Spinosad), Chlorpyrifos and Methoxyfenozide® increased the cotton yield. El-Naggar (2009) reported that the application of Easterna followed by Super Biovert and Spinosad in respect increased the cotton yield compared to the untreated control in both seasons of 2006 and 2007. Meanwhile, he found that sprays of Easterna/Greenzit S.P₁₀₀/Spinosad only increased cotton yield in season 2007. Similar results were also explained by Sun and Xu (1986), El-Naggar (1998), Mesbah *et al.* (2000) and El-Naggar *et al.* (2008).

Effect on the activity of larval protease on the spiny bollworm: As indicated in Table 4 the tested foliar fertilizers and all alternative chemical compounds had severed effects on the activity of larval protease on the spiny bollworm, *E. insulana* in comparison to untreated check. The detected differences in enzyme activity occurred after larval exposure to the applied sequences of these compounds. In this respect, the foliar treatments of Chlorpyrifos and EAP/Radiant®/

Table 4: Effects of foliar sprays on the activity of larval protease of *Earias insulana*

Treatments	Activity (OD/mg protein/min)
EAP/Greenzit® SP ₁₀₀ /Ascobin®	0.369
EAP/Greenzit® SP ₁₀₀ /Radiant®	0.567
EAP/Greenzit®SP ₁₀₀ /Chlorpyrifos	0.482
EAP/Radiant®/methoxyfenozide	0.672
Radiant®/Methoxyfenozide/Chlorpyrifos	0.283
Chlorpyrifos	0.697
Untreated check	0.194

Methoxyfenozide® gave the highest value of enzyme activity which amounted to 0.697 and 0.672 OD/mg protein/min, respectively, followed by the treatment of EAP/Greenzit®S.P₁₀₀/Radiant® (0.567 OD/mg protein/min) compared to untreated check (0.194 OD/mg protein/min). On the contrary, the treatments of Radiant®/Methoxyfenozide®/Chlorpyrifos decrease the enzyme activity to the lowest value (0.283 OD/mg protein/min). On the other hand, the other applied foliar treatments gave somewhat higher enzyme activity than the untreated check and ranged from 0.369 in case of EAP/Greenzit® S.P₁₀₀/Ascobin® to 0.482 larvae/10 bolls in case of EAP/Greenzit® S.P₁₀₀/Chlorpyrifos.

CONCLUSION

It could be concluded that the results of the present investigation indicated that the foliar treatments of EAP/Radiant®/ methoxyfenozide® and Radiant®/methoxyfenozide®/Chlorpyrifos gave the highest value and lowest value of Protease enzyme activity, respectively but in the same time, these treatments gave the same highly efficient on the spiny bollworm infestation and gave higher increase cotton yield.

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