EFFICACY OF SOME FOLIAR FERTILIZERS AND ALTERNATIVE CHEMICALS ON THE SPINY BOLLWORM, *EARIAS INSULANA* (BOISD.) LARVAE (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The present study was carried out on cotton variety Giza 70 during two consecutive seasons of 2006 and 2007 in the Experimental Farm of the regional research station, Alexandria, Egypt. The study was to evaluate two foliar nutrients, a plant growth promotor (PGP) and two alternative chemical compounds in addition to a standard insecticide (Chlorpyrifos). All was done to determine the possible use of the above compounds in a program of Integrated Pest Management (IPM) of the spiny bollworm, Earias insulana (Boisd.) beside the effect of each on larval DNA structure and on cotton yield.

The obtained results indicated that the highest efficacy of the following foliar treatment with Easterna Aminofert / Greenzit S.P$_{100}$ / Spinosad have decreased the incidence of spiny bollworm, E. insulana infestation in cotton season 2006. The foliar treatments of Easterna. / Greenzit S.P$_{100}$ with Spinosad or / and Chlorpyrifos were also high efficient in reducing the level of spiny bollworm infestation in season 2007.

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, sprays of Easterna / Greenzit S.P$_{100}$ / Spinosad only increased cotton yield in season 2007.

The consequent treatment of Easterna /GreenzitS.P$_{100}$ / Methoxyfenozide and / or Easterna. /Super Biofert / Methoxyfenozide, severely affected the DNA structure of Earias insulana larvae in both seasons.

INTRODUCTION

The cotton bollworms *Earias insulana* (Boisd.) (Lepidoptera: Noctuidae) are considered to be the most destructive late season insect-pests of cotton plant; the highly preferred host for these insects in Egypt. The indiscriminating use of insecticides has caused a number of ecological, economical and social hazardous problems to various ecological agro-ecosystems around the world including Egypt. Besides, the resistance to pesticides appeared in several insect pests. Therefore, the results from this investigation could be attributed to the possibly right use of each bio- or / and foliar fertilizers as control mean in the Integrated Pest Management (IPM) of the spiny bollworm, *E. insulana* to avoid the increasing use of conventional insecticides and reduce the occurring environmental pollution.

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 of disease and insect 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 and Mesbah *et al.*, 2000).

Therefore, the present study was adopted to evaluate certain treatments of foliar nutrients, a Bio-insecticide (Spinnosad or Spintor 24 SC®), an Insect Growth Inhibitor (Methoxyfenozide or Runner®), Plant Growth Promotor (PGP) and Organophosphorous insecticide (Chlorpyrifos) in different sequences to minimize spiny bollworm, *Earias insulana* (Boisd.) injury to the plant; and to study their probable side effect on DNA structure and cotton yield.

MATERIAL AND METHODS

Field experiments were carried out at the experimental farm of regional research station, plant protection Res. Instit.; Agric. Res. Centre, Alexandria Governorate throughout the two successive cotton growing seasons of 2006 and 2007. Whereas, in both seasons an area of half feddan was cultivated with cotton variety "Giza 70" on May 28th and 24th April, respectively. 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). 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 2006 and 2007, prior to sewing, the cotton seeds were dressing with the bio-fertilizer Miicrobin®. The divided experimental area included 6 foliar treatments in sequences beside the control. The sequences included two foliar nutrients, a Bio-insecticide (Spinosad), an insect growth inhibitor (Methoxyfenozide), a plant growth promotor (Super Biofert) and Chlorpyrifos as an insecticide as 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 spiny bollworm, *E. insulana*, cotton yield and larval DNA structure. Sprayings were performed using knapsack sprayer (20 L).

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1- Estimation of spiny bollworm infestation:

Weekly inspections were done to determine the infestation levels of spiny bollworm. Samples of 10 green bolls / plots were taken randomly; examined bolls for each treatment were 30 green bolls. The weekly estimation of bollworms 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. Statistical analysis using "F" and "L.S.D" tests for the comparison and evaluation of the tested treatments were used.

2 - 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.

3 - Effect on larval DNA structure

All larvae were used to extract DNA by adding sufficient amount of liquid nitrogen and then the all larvae were crushed. The method described by Sambrook *et al.*, (1989) and Pitheret *et al.*, (1989) for DNA isolation was used. The resulting DNA pellet from whole used larvae was washed with 70 % ethanol, centrifuged at 8,000 rpm for 30 min at 4°C and air dried. DNA pellet was then dissolved in TE buffer. "TE" is derived from its components: Tris (hydroxymethyl), and EDTA (ethylenediaminetetraacetic acid), a molecule chelating cations like Mg^{2+} (10 ml Tris, bring to pH 7.5 with HCl , 1 ml M EDTA), where is a commonly used buffer solution in molecular biology, especially in procedures involving DNA or RNA.. The purpose of TE buffer is to protect DNA or RNA from degradation. Genomic DNA was analyzed and visualized by agarose gel according to the method of Sambrook *et al.*, (1989). The gel was prepared with 0.8 % (w/v) agarose dissolved in TBE [54 gm tris + 27.5 gm boric acid + 20 ml of 0.5 M EDTA (pH 8)]. The run was performed at 77 volt. The gels were stained with Sybr® Green (Katl GmbH- Germany). Genomic DNA was restricted with *Eco*R1 according to the instructions described by Sambrook *et al.*, (1989). Southern blot for hybridization was carried out using Hybrid™N^+ (positively charged nylon membrane), (Merkel, Germany) so as to illustrate the localization of the affected regions with regard to the used treatments.

Tested compounds

1- Easterna Aminofert Plus (Liquid) is produced by Easterna Company for Agriculture Development (Easterna , Egypt). The main components are: amino acids (10%), saccarides and its related (6%), nitrogen (6%), phosphorous (4%), potassium (6%), sulphur (5%), organic matter (85 %), magnesium (5000 ppm), chelated ferric (1000 ppm), chelated zink (500 ppm), chelated manganese (250 ppm), chelated cupper (150 ppm), boron (100 ppm).

2- Greenzit SP_{100} is produced by Ciba Geigy limited, (Basal, Swiss) and the main components are: EDTA Na_2Mn (40 %) and EDTA Na_2Zn (43 %) supplemented by Ca (0.054), Fe (5.40), Mo (0.027) Mg (0.54) Ni (0.005) Mn (5.54) Cu (0.005) Zn (70.27) gm / Kg.

Super Biofert (PGP):

It is also produced by Easterna Company for Agriculture Development (Easterna , Egypt). The main components are: amino acids (10%), Potassium glutamate (8%), saccarides and its related (6%), organic acid (growth promotor) (10%), organic phosphorous (4%), magnesium citrate (1%), saccharomyces fortified with vitamins (5%) and chelated elements 0.2%.

Spinosad (Spintor 24% SC):

It is a metabolite of the Actinomycete, *Saccharopolyspora spinosa* Martz & Yao. It is a naturally occurring mixture of two active products (Spinosyn A & D). It is a trademark of Dow Agro Sciences Co (Dow, England).

Insect Growth Inhibitor (IGI)

Methoxyfenozide or Runner® 24% (SC); N-tert-butly-N-(3,5-dimethylbenzoyl)-3-methoxy-2-methylbenzohydrazide. It was supplied by Rohm and Hass Co (Dow, England).

Organophosphorous compound:

Chlorpyrifos or Dursban® 48 % (EC); 0,0 diethyl 0-3,5,6 trichloro-2-pyridyl phosphorothioate (Dow, England).

RESULTS AND DISCUSSION

1- Efficacy of treatments on spiny bollworm infestation

A – Season of 2006

The illustrated results in Table (2) are expressed as mean numbers of detected spiny bollworm larvae per ten of bolls. The consequent foliar treatment of Easterna. / Greenzit $S.P_{100}$ / Spinosad gave the lowest level of spiny bollworm infestation (1.03 larvae / 10 bolls) compared with the untreated check (1.7 larvae / 10 bolls). Comparatively, for the other tested foliars sequences, more or less higher means of infestation were obtained but still less than the untreated control and the application of consequence Easterna / Super Biofert / Spinosad (1.7 larvae / 10 bolls) that are adjusted from 1.23 in case of Easterna / Greenzit $S.P_{100}$ / Methoxyfenozide ® to 1.57 larvae / 10 bolls in case of Easterna / Super Biofert / Methoxyfenozide ®.

B- Season of 2007

Table (3) shows the effect of the foliar treatments on the mean numbers of inspected spiny bollworm larvae / 10 bolls. From the table, in compared to the untreated control (1.97 larvae / 10 bolls), it could be noticed that each of the treatments of Easterna / Greenzit $S.P_{100}$ / Spinosad, Easterna / Greenzit $S.P_{100}$ / Chlorpyrifos and Easterna / Super Biofert / Spinosad were highly efficient and gave the lowest level of spiny bollworm infestation not exceeding 0.9 larvae / 10 bolls. This was followed by the sequences of Easterna / Greenzit $S.P_{100}$ / Methoxyfenozide ® and Easterna / Super Biofert / Chlorpyrifos which gave somewhat higher infestation levels of spiny bollworm but less than the control (1.3 and 1.37 larvae / 10 bolls, in respect). Meanwhile, the foliar application of Easterna / Super Biofert / Methoxyfenozide ® was the least efficient (1.77 larvae / 10 bolls).

The above cited results are ascertained by the concluded results by 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 $S.P_{100}$ have also lowered spiny bollworms infestation. Also, the obtained results by Putothit and Deshpand (1994) and Mesbah *et al.* (2000) were in full agreement with the present aforementioned and illustrated findings.

2 - Efficacy of tested foliars on cotton yield in seasons of 2006 and 2007.

From Table (4), it could be observed that the application of Easterna / Super Biofert / Spinosad gave the highest cotton yield increase (188.4 %), versus, the applied foliars sequence of Easterna / Super Biofert / Chlorpyrifos which gave the lowest increase (7.2 %). For the other applied foliar treatments, the estimated increase of cotton yield was, to a more or less extent, lowered but still higher than the untreated control and ranged from 21.7 to 59.4 % in season of 2006.

Similar results were noticed in the growing cotton season of 2007. The sequence of Easterna / Super Biofert / Spinosad also gave the highest increase of cotton yield in comparison to the control (197.7 %), followed by Easterna / Greenzit $S.P_{100}$ / Spinosad (143.4 %). For the other tested foliar treatments, the estimated increase of cotton yield was lower, but still higher than the untreated check and ranged from 13.04 % for Easterna / Super Biofert / Chlorpyrifos to 65.2% for Easterna / Greenzit $S.P_{100}$ / Methoxyfenozide ®. These 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 $S.P_{100}$, 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. Similar results were also explained by Sun and Xu (1986), EL-Naggar (1998) and Mesbah *et al.* (2000).

3 - Efficacy of tested foliars on DNA structure of treated larvae

It is clear from the exhibited results in Figures (1 & 2) that the tested foliar fertilizers and all alternative chemical compounds in both seasons, (2006 and 2007) had sever effects on the DNA structure in larvae of the spiny bollworm, *E .insulana* where several DNA bands were visualized for the larval DNA. The number and intensity of bands differed in response to the performed treatments of foliars and other alternative compounds with induced DNA fragmentations in different sites of the larval DNA where compared with the control

The detected changes in DNA size of larvae may be due to the fragmentation occurred after larval exposure to the applied sequencies of these compounds. In this respect, no DNA damage was observed in the control. For Easterna /Greenzit $S.P_{100}$ / Methoxyfenozide and Easterna /Super Biofert/ Methoxyfenozide sequencies, the intensity of some bands was decreased and altered; therefore it is suggested that these foliar sequenceies were more effective on the synthesis of larval DNA. On the contrary, slight changes occurred in the patterns of other applied foliar sequencies. Moreover, these evaluated foliar fertilizers and alternative compounds may have marked effects on the larval proteins synthesis of *E. insulana* over time due to the use of these sequencies in both seasons of 2006 and 2007.

It is suggested that those compounds may bind to DNA through the reactive groups of their active moieties, leading to destabilization as well as unwinding of the DNA. This could be a possible mechanism for its genotoxicity (Saxena *et al.*, 2005); or may induce oxidative stress and generation of reactive oxygen species (ROS) in larval systems causing larval DNA damage (Zegura *et al.*, 2004). These results are similar to the findings reported by Mukhopadhyay *et al.*, (2004), where the larvicide they used caused DNA damage in brain ganglia of *Drosophila melanogaster*. Full agreement with the present results, where found when Cypermethrin induced free radical-mediated lipid peroxidation in rat brain. This provides evidence that the observed DNA damage after Cypermethrin exposure could be a consequence of free radical attack to DNA (Mukhopadhyay *et al.*, 2004 & Gad and Abdel-Megeed, 2006).

Table (1): Tested foliars and their rates of application during the growing seasons of 2006 and 2007.

Treatments	Rate of application / L
Easterna Aminofert /GreenzitS.P_{100} / Spinosad	10 ml / 0.2 g / 0.3 ml
Easterna Aminofert /GreenzitS.P_{100} / Methoxyfenozide	10 ml / 0.2 g / 0.5 ml
Easterna Aminofert /GreenzitS.P_{100} / Chlorpyrifos	10 ml / 0.2 g / 5 ml
Easterna Aminofert /Super Biofert / Chlorpyrifos	10 ml / 1.25 ml / 5 ml
Easterna Aminofert /Super Biofert/Spinosad	10 ml / 1.25 ml / 0.3 ml
Easterna Aminofert /Super Biofert/ Methoxyfenozide	10 ml / 1.25 ml / 0.3 ml

Table (2): Effects of tested foliar treatments on the mean numbers of spiny bollworm larvae / ten bolls throughout the growing season of 2006.

Treatments	Mean numbers of larvae / 10 bolls
Easterna. / Greenzit S.P_{100} / Spinosad	1.03 c
Easterna. / Greenzit S.P_{100} / Methoxyfenozide	1.23 bc
Easterna. / Greenzit S.P_{100} / Chlorpyrifos	1.4 abc
Easterna. / Super Biovert / Chlorpyrifos	1.53 ab
Easterna. / Super Biovert / Spinosad	1.7 bc
Easterna. / Super Biovert / Methoxyfenozide	1.57 ab
Control	1.7 a
Significance	High

Fcalculated = 3.07 D.f = 6 L.S.D.05 = 0.384 P = 0.0078
Means followed by the same letter are not significantly different at the 5 % level

Table (3): Effects of foliar sprays on the mean numbers of *E. insulana* larvae / ten bolls throughout the growing season of 2007.

Treatments	Mean numbers of larvae / 10 bolls
Easterna. / Greenzit S.P_{100} / Spinosad	0.6 c
Easterna. / Greenzit S.P_{100} / Methoxyfenozide	1.3 b
Easterna. / Greenzit S.P_{100} / Chlorpyrifos	0.77c
Easterna. / Super Biovert / Chlorpyrifos	1.37 b
Easterna. / Super Biovert / Spinosad	0.9 c
Easterna. / Super Biovert / Methoxyfenozide	1.77 a
Control	1.97 a
Significance	High

Fcalculated = 18.26 D. f = 6 L.S.D.05 = 0.335 P. = 0.0000
Means followed by the same letter are not significantly different at the 5 % level

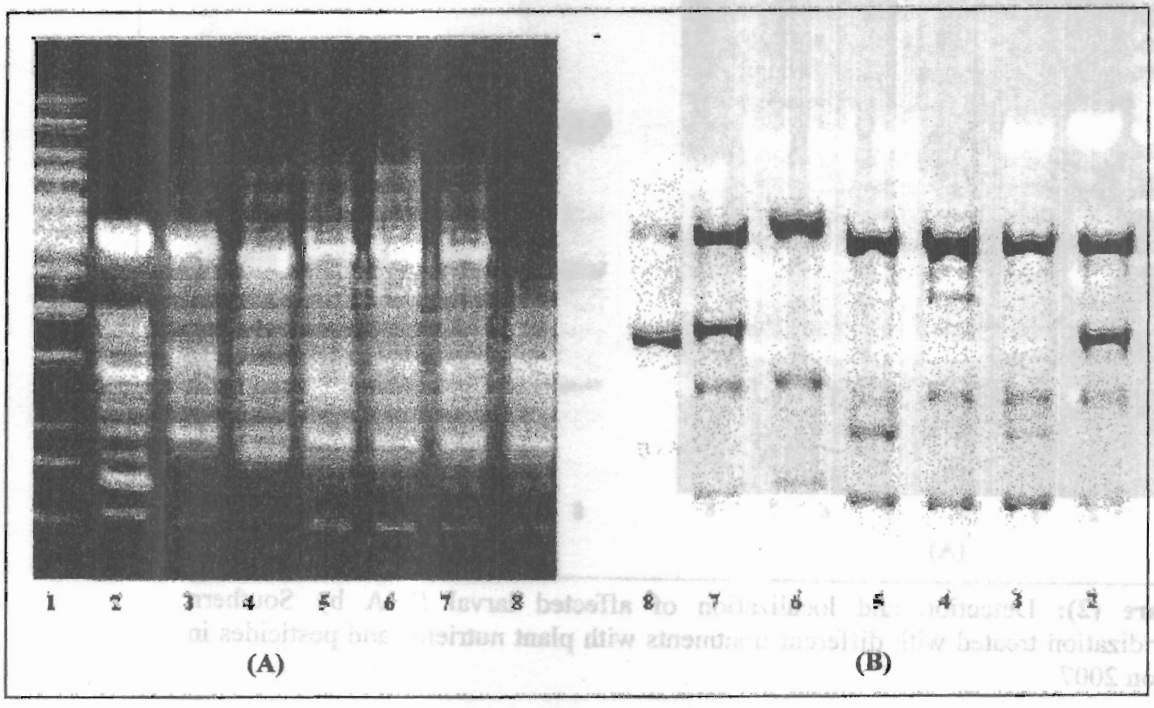

Figure (1): Detection and localization of affected larval DNA by Southern hybridization related to different treatments with plant nutrients and pesticides in season 2006.

Lane 1: Marker 10,000 bp
- **2:** Control
- **3:** Easterna Aminofert /Greenzit$S.P_{100}$ / Spinosad
- **4:** Easterna. / Greenzit $S.P_{100}$ / Methoxyfenozide
- **5:** Easterna. / Greenzit $S.P_{100}$ / Chlorpyrifos
- **6:** Easterna. / Super Biovert / Chlorpyrifos
- **7:** Easterna. / Super Biovert / Spinosad
- **8:** Easterna. / Super Biovert / Methoxyfenozide

❖ Agarose gel electrophoresis (1.25%) showing the digestion of insect genomic DNA with *Eco*R1

❖ (B) Southern hybridization analysis of the localizations of total DNA in positive charged nylon membrane and probed total DNA for explanation of the DNA affected regions regarding treatments

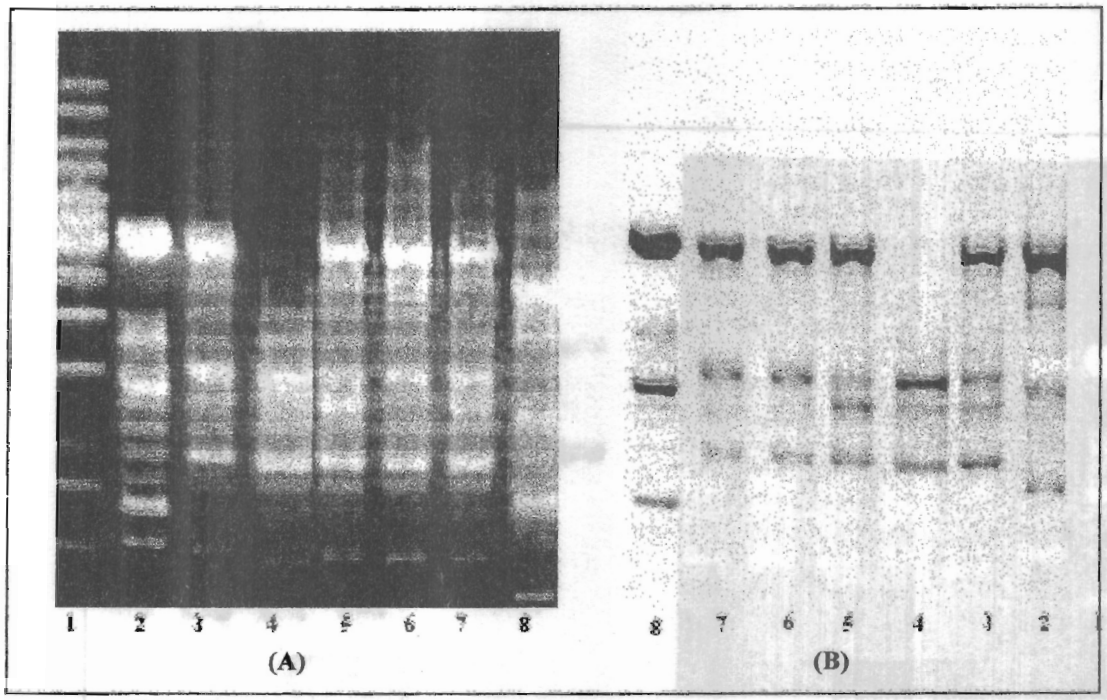

Figure (2): Detection and localization of affected larval DNA by Southern hybridization treated with different treatments with plant nutrients and pesticides in season 2007.

Lane 1: Marker 10,000 bp
Lane 1: Marker 10,000 bp
2: Control
3: Easterna Aminofert /GreenzitS.P_{100} / Spinosad
4: Easterna. / Greenzit S.P_{100} / Methoxyfenozide
5: Easterna. / Greenzit S.P_{100} / Chlorpyrifos
6: Easterna. / Super Biovert / Chlorpyrifos
7: Easterna. / Super Biovert / Spinosad
8: Easterna. / Super Biovert / Methoxyfenozide

- Agarose gel electrophoresis (1.25%) showing the digestion of insect genomic DNA with *Eco*R1
- **(B)** Southern hybridization analysis of the localizations of total DNA in positive charged nylon membrane and probed total DNA for explanation of the DNA affected regions regarding treatments

Differences in the observed DNA damage in lanes 4 and 8 shown in Figures (1 & 2) could be explained by the fact that there is a differential expression of basal levels of DNA repair genes in various tissues of the *E. insulana* larval DNA. Generally, from the obtained results, it can be concluded that the treatment with the foliar sequencies of Easterna /GreenzitS.P_{100} / Methoxyfenozide and Easterna /Super Biofert/ Methoxyfenozide, were the most effective than other tested sequencies on DNA of *E. insulana* larvae.

It could be concluded that the results of this investigation indicated that the foliar treatment of Easterna followed by Greenzit S.P_{100} and Spinosad decreased the incidence of spiny bollworm infestation in the growing seasons of 2006 and 2007. Moreover, the sequent application of Easterna / Super Biovert / Spinosad gave the highest increase of cotton yield in the seasons of 2006 and 2007. The treatments of Easterna /GreenzitS.P_{100} / Methoxyfenozide and Easterna /Super Biofert / Methoxyfenozide, have severely affected *E. insulana* DNA structure of larvae in both seasons of 2006 and 2007.

Table (4): Effects of foliar sprays on the cotton yield during the growing seasons of 2006 and 2007.

Sequences	2006		2007	
	Weight / feddan Kg (ken.)	% Increase*	Weight / feddan Kg (ken.)	% increase*
Easterna. / Greenzit S.P_{100} / Spinosad	733.3 kg (4.6 ken.)	59.4 %	746.7 kg (4.7 ken.)	143.4 %
Easterna. / Greenzit S.P_{100} / Methoxyfenozide	693.3 kg (4.4 ken.)	50.7 %	506.7 kg (3.2 ken.)	65.2 %
Easterna. / Greenzit S.P_{100} / Chlorpyrifos	560 kg (3.5 ken.)	21.7 %	360 kg (2.28 ken.)	17.3 %
Easterna. / Super Biovert / Chlorpyrifos	493.3 kg (3.1 ken.)	7.2 %	346.7 kg (2.20 ken.)	13.04 %
Easterna. / Super Biovert / Spinosad	1326.7 kg (8.42 ken.)	188.4 %	913.3 kg (5.7 ken.)	197.7 %
Easterna. / Super Biovert / Methoxyfenozide	693.3 kg (4.4 ken.)	50.7 %	474.7 kg (3.01 ken.)	54.7 %
Control	460 kg (2.9 ken.)		306.7 kg (1.9 ken.)	

* expressed as % of increase than the untreated check, according to Hussein *et al.* (2002).
Kentar= 157.5 Kg

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الملخص العربى

فعالية بعض الأسمدة الورقية والبدائل الكيماوية على يرقات دودة اللوز الشوكية

على زكريا النجار

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الإسكندرية – مصر

تم إجراء الدراسة على صنف قطن جيزة ٧٠ فى موسمين متتالين ٢٠٠٦ ، ٢٠٠٧ فى المزرعة البحثية لمحطة البحوث الإقليمية – الإسكندرية وذلك بهدف تقييم مركبين من الأسمدة الورقية ، ومركبين من بدائل المواد الكيماوية بالإضافة إلى المبيد الحشرى (كلوروبيرفوس) ، ومنظم نمو نباتى ومدى إمكانية إستخدامهم فى برنامج المكافحة المتكاملة لدودة اللوز الشوكية ودراسة تأثيرها على كل من التغيرات فى DNA فى اليرقات ، وعلى إنتاجية محصول القطن.

أوضحت النتائج أن أعلى تأثير على الإصابة بدودة اللوز الشوكية تم الحصول عليه عند إستخدام التتابع إسترنا أمينو فيرت / جرينزيت إس بى ١٠٠ / سبينوساد حيث إنخفضت نسبة الإصابة بدرجة كبيرة وذلك فى موسم ٢٠٠٦. بينما أعطت المعاملات إسترنا أمينو فيرت / جرينزيت إس بى ١٠٠ مع سبينوساد أو / مع كلوروبيرفوس تأثيرا عاليا فى خفض الإصابة بدودة اللوز الشوكية فى موسم ٢٠٠٧.

أعطى التتابع التالى إسترنا أمينو فيرت / سوبر بيوفيرت / سبينوساد أعلى زيادة فى محصول القطن مقارنة مع الكنترول فى كل من الموسمين ٢٠٠٦ ، ٢٠٠٧. بينما فى موسم ٢٠٠٧ أعطت المعاملة إسترنا أمينو فيرت / جرينزيت إس بى ١٠٠ / سبينوساد أعلى زيادة فى محصول القطن.

أحدثت كل من التتابعات التالية إسترنا أمينو فيرت / جرينزيت إس بى ١٠٠ / ميثوكسى فينوزايد و/ أو إسترنا أمينو فيرت / سوبر بيوفيرت / ميثوكسى فينوزايد تأثيرا ضارا فى تركيب DNA فى يرقات دودة اللوز الشوكية فى الموسمين.