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New Measures for the Control of Cotton Bollworms *Pectinophora gossypiella* (Lep.: Gelechiidae) and *Earias insulana* (Lep.: Noctuidae), with Reference to the Side Effect on Certain Soil Enzymes

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ABSTRACT

Field experiments were carried out at the Agricultural Research Experimental Farm, Alexandria Governorate, Egypt to evaluate two foliar nutrients, a plant growth promoter (PGP) and two alternative chemical compounds in addition to two standard insecticides (Chlorpyrifos and Spinosad) to determine their possible use in an Integrated Pest Management (IPM) program of the pink bollworm, *Pectinophora gossypiella* and the spiny bollworm, *Earias insulana* infestations. The side effects of these compounds on certain soil enzymes as well as their efficacy were monitored on cotton yield during the 2006 and 2007 growing seasons. The lowest infestation value of pink bollworm was induced by Easterna Aminofert / Greenzit S.P_{100} with Chlorpyrifos and Spinosad in the 2006 season and by the Easterna Aminofert / Greenzit S.P_{100} / Spinosad sequence in the 2007 season. For the spiny bollworm, the sequence of Easterna Aminofert / Greenzit S.P_{100} / Spinosad gave the lowest infestation percentage in the 2006 season, which, in the 2007 growing season, was obtained when Easterna Aminofert / Greenzit S.P_{100} with Spinosad and Chlorpyrifos and Easterna Aminofert / Super Biovert / Spinosad were used. The foliar application of Easterna Aminofert / Super Biovert / Spinosad resulted in a higher cotton yield in both seasons, estimated at a 201.1 and 214.2% increase, respectively, followed by Easterna Aminofert / Greenzit S.$P_{100\,/}$ Spinosad. The sequences of Easterna Aminofert / Super Biovert with Spinosad and Methoxyfenozide and Easterna Aminofert / Greenzit S.P_{100} / Methoxyfenozide decreased urease activity while a foliar application of Easterna Aminofert / Super Biovert / Spinosad and Easterna Aminofert / Greenzit S.P_{100} / Spinosad showed the lowest dehydrogenase activity. In general, dehydrogenase was more influenced by the evaluated sequences than urease.

Keywords: chloropyriphos, dehydrogenase, foliar nutrients, sequences, spinosad, urease

INTRODUCTION

Cotton growers in Egypt have experienced severe economic losses from cotton pests due to reduced yields, low lint quality and increased costs of insecticides (Amin and Gergis 2006). Insect-pests are considered one of the important factors that influence cotton production and cause economical damage to the crop yield. Damage causing losses to fruit is frequently more destructive than that to leaves, stems and roots. Profitable cotton production in Egypt depends on successful and efficient pest management programmes which reduce the disaster of crop losses particularly caused by insect-pests. Pink bollworm, *Pectinophora gossypiella* (Saunders) and the spiny bollworm, *Earias insulana* (Boisd) are the most serious cotton pests in Egypt (Hussein *et al*. 2002). They cause the greatest yield losses from nearly one million hectares cultivated annually (Haque 1991; El-Naggar 1998). The spiny bollworm larvae damage buds early in the growing season and squares, and bolls later in the season. Various control methods mainly the conventional chemical insecticides are used for pest control in agriculture. Careless or excessive use of pesticides often results in poor control due to their drastic effect on natural enemies, besides the higher expenses and hazards to health and the environment (Atwal 1994). On the other hand, the misuse of pesticides might also accelerate the occurrence of resistance in many of insect species (Amin and Gergis 2006). In addition, overpollution of the environment, toxic residues, problems and carcinogenicity of these insecticides are well known.

Therefore, using new approaches in pest management systems is highly encouraged. Foliar nutrient sprays may be a good tool to produce a profitable cotton crop that can compete with weeds and is able to outgrow and overcome disease and insect damage. To prevent this problem, there is a need for different trends in minimizing insecticides incorporated with foliar nutrients and plant growth promoter PGP (substances used for better management of nutrients and plant growth) to maintain the viability and vitality of the plants (Beanland *et al.* 2003). Foliar sprays can also correct deficiencies of certain macro- and micronutrients (Krantz and Nelsted 1964; Mesbah *et al*. 2000).

This work was undertaken to evaluate some proposed new biologically-based and area-wide sequences for management of key cotton pests in Egypt. Moreover, this study directly aimed to evaluate the effects of these compounds on soil urease and dehydrognase as well as cotton yield.

MATERIALS AND METHODS

Field experiments were conducted during the 2006 and 2007 growing seasons at the Agricultural Research Experimental Farm, Agricultural Research Center, Alexandria Governorate, Egypt. An area of a half feddan (2100 m^2) was planted with cotton variety 'Giza 70' on the 28th May, 2006 and on the 24th April, 2007.

The experimental area was divided into plots (0.01 feddan each). Each plot was separated from the adjacent one by a half meter belt (barrier) to minimize the interference of spray drift from one treatment to another. The experimental design was a split-plot with three replicates for each treatment during each season. The

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sown cotton seeds were treated with the bio-fertilizer Microbin® (micronutrient spreader attachment and an add-on hopper that allows spreading granular micronutrients or seeds as a second or third product; Phytopharma Ltd., India). The experimental area included 6 sequences. The sequences included three foliar fertilizers: Super Biovert (Phytopharma), Easterna Aminofert (Easterna Co. for Agriculture Development, The Netherlands), Greenzit SP_{100} (Ciba Geigy Ltd.), Basal (Novartis). Methoxyfenozide (KZ Co., Egypt; used as PGP), Spinosad (KZ), and Chlorpyrifos (Syngenta) were the insecticides used (**Table 1**).

The sequences of these compounds were applied at three periods (5, 10 and 15 days); at the beginning of flowering, after 50% flowering and at the beginning of the fruiting stage. Throughout both seasons, inspections were carried out to determine the effect of the tested compounds on the population of bollworms (pink and spiny), cotton yield and soil enzymes (urease and dehydrogenase). Sprayings were performed using a knapsack sprayer (20 L). The untreated ones (i.e. treatments without the tested compounds and sequences) were carried out in parallel to monitor the differences among the treatments.

Estimation of spiny and pink bollworm infestation

Weekly inspections were done to determine the infestation levels with pink bollworm (*Pectinophora gossypiella* Saunders) and spiny bollworm (*Earias insulana* Boisduval). To determine the infestation level with bollworms, weekly samples of 10 green bolls/ plots were taken randomly: 30 green bolls were examined for each treatment. The level of infestation by the bollworms was estimated weekly over 10 weeks during the following seasons. In each sample, the bolls were examined externally before dissection and internal inspection. Infestation records were based on the existence of injury symptoms regardless the presence of the larvae.

Determination of cotton yield

To estimate the cotton yield/plant/feddan in each treatment, ripe bolls from 25 cotton plants were collected, weighed and air dried for 10 h and data were used to calculate total yields.

Assay of soil enzymes

Soil samples

To assay soil enzymes, 5 random samples (150 g) and 3 replicates from each treatment were taken around (15 cm diameter) each plant (20-30 cm depth); afterwards they were mixed homogeneously and transferred to the laboratory to be analyzed. The samples were taken after 5, 10 and 15 days from the beginning of each treatment.

Dehydrogenase activity assay

Dehydrogenase activity in soil was determined according to the reduction of 2,3,5- tripheneyltetrazolium chloride (TTC) to triphenyl formazan (TPF) which is extracted and measured spectrophotometrically at 420 nm (Casida *et al*. 1964).

Urease activity assay

Urease activity was calorimetrically determined at 485 nm according to the method described by Lambert *et al*. (2004), where urea content were expressed as μg urea/g air-dried soil. Urease activity was determined as:

$$\text{Urease activity \%} = \frac{A - B}{A} \times 10$$

where
A is the original urea added in ppm.
B is the remaining urea (residues) in ppm.
A-B = the converted amount of urea in ppm.

Tested compounds

The compounds were applied individually and consequently in a sequence.

Easterna Aminofert Plus (Liquid) is produced by Easterna Co. for Agriculture Development (The Netherlands). The main components are: amino acids (10%), saccharides and its related compounds (6%), N (6%), P (4%), K (6%), S (5%), organic matter (85%), Mg (5000 ppm), chelated ferric (Fe/amino acids chelate) (1000 ppm), chelated zinc (Zn/amino acid chelate) (500 ppm), chelated manganese (Mn-EDTA) (250 ppm), chelated cupper (Cu-EDTA) (150 ppm), Bo (100 ppm).

Greenzit SP_{100} is produced by Ciba Geigy limited, Basal (Novartis) 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) g/Kg.

Super Biofert (PGP) is also produced by Easterna Company for Agriculture Development (Egypt). The main components are: amino acids (10%), potassium glutamate (8%), saccharides and their related compounds (6%), organic acid (growth promoter) (10%), organic phosphorous (4%), magnesium citrate (1%), *Saccharomyces cerevisiae* fortified with vitamins (5%) and chelated elements 0.2%.

Spinosad or Spintor® (24% soluble concentrate (SC), Dow Agro Sciences Co. (USA)) is a metabolite of the actinomycete, *Saccharopolyspora spinosa*. It is a naturally occurring mixture of two active products (Spinosyn A and D). It is a trademark of Dow Agro Sciences Co.

Methoxyfenozide or Runner® 24% (SC); (China) *N*-tert-butyl-*N*-(3,5-dimethylbenzoyl)-3-methoxy-2-methylbenzohydrazide, is an insect growth inhibitor (IGI). It was supplied by Rohm and Haas Co., Mozzanica, Italy.

Chlorpyrifos or Dursban® (48% as emulsified concentrate (EC); (Indian Pesticides Ltd., Global Agrochemicals Co.) O,O-diethyl O-3,5,6 trichloro-2 pyridyl phosphorothioate is an organophosphorous compound.

Statistical analyses

Data obtained were subjected to the analysis of variance and the means were compared using the LSD test at $P < 0.05$.

RESULTS

Efficiency of foliar applications on the pink and spiny bollworm in two growing seasons

A. Effects on pink bollworm

Table 2 indicates the calculated average of inspected numbers of pink bollworm in the 2006 and 2007 growing seasons. The performed sequent sprays of Easterna Aminofert / Greenzit $S.P_{100}$ / Chlorpyrifos and Easterna Aminofert / Greenzit $S.P_{100}$ / Spinosad significantly decreased pink bollworm infestation (0.03 and 0.07 larvae/10 bolls, respec-

Table 1 The tested sequences compounds (foliar biofertilizers, PGP and pesticides) and application rates during the 2006 and 2007 growing seasons.

Treatments	Rate of application/L
1. Easterna Aminofert / Greenzit SP_{100} / Spinosad	10 ml / 0.2 g / 0.3 ml
2. Easterna Aminofert / Greenzit SP_{100} / Methoxyfenozide	10 ml / 0.2 g / 0.5 ml
3. Easterna Aminofert / Greenzit SP_{100} / Chlorpyrifos	10 ml / 0.2 g / 5 ml
4. Easterna Aminofert / Super Biovert / Chlorpyrifos	10 ml / 1.25 ml / 5 ml
5. Easterna Aminofert / Super Biovert / Spinosad	10 ml / 1.25 ml / 0.3 ml
6. Easterna Aminofert / Super Biovert / Methoxyfenozide	10 ml / 1.25 ml / 0.3 ml

Table 2 Effects of tested compounds on the mean numbers of pink bollworm infestation/10 bolls throughout the 2006 and 2007 growing seasons.

Treatments	Mean № of larvae / 10 bolls	
	2006	2007
1. Easterna Aminofert / Greenzit SP_{100} / Spinosad	0.07 b	0.63 c
2. Easterna Aminofert / Greenzit SP_{100} / Methoxyfenozide	0.23 ab	1.07 b
3. Easterna Aminofert / Greenzit SP_{100} / Chlorpyrifos	0.03 b	1.13 ab
4. Easterna Aminofert / Super Biovert / Chlorpyrifos	0.20 ab	1.13 ab
5. Easterna Aminofert / Super Biovert / Spinosad	0.20 ab	1.17 ab
6. Easterna Aminofert / Super Biovert / Methoxyfenozide	0.17 ab	1.10 b
Untreated check	0.37 a	1.47 a
Significance	**	**
F calc.	2.67	4.45
F tab.	2.23	2.23
$L.S.D._{.05}$	0.188	0.324

Means followed by the same letter(s) are not significantly different at the 5% level

Table 3 Effects of performed foliar sprays on the mean numbers of spiny bollworm larvae/10 bolls throughout the 2006 and 2007 growing seasons.

Treatments	Mean numbers of larvae / 10 bolls	
	2006	2007
1. Easterna Aminofert / Greenzit SP_{100} / Spinosad	1.03 c	0.60 c
2. Easterna Aminofert / Greenzit SP_{100} / Methoxyfenozide	1.23 bc	1.30 b
3. Easterna Aminofert / Greenzit SP_{100} / Chlorpyrifos	1.40 abc	0.77 c
4. Easterna Aminofert / Super Biovert / Chlorpyrifos	1.53 ab	1.37 b
5. Easterna Aminofert / Super Biovert / Spinosad	1.17 bc	0.90 c
6. Easterna Aminofert / Super Biovert / Methoxyfenozide	1.57 ab	1.77 a
Untreated check	1.70 a	1.97 a
Significance	**	**
F. calc.	18.26	3.07
F. tab.	2.23	2.23
$L.S.D._{.05}$	0.335	0.384

Means followed by the same letter(s) are not significantly different at the 5% level

tively), while the other sequences resulted in a higher infestation which ranged from 0.17 to 0.23 larvae/10 bolls in comparison to the untreated check (0.037 larvae/10 bolls) in the 2006 growing season.

Moreover, there was a significant decrease in the infestation of pink bollworm occurring in the 1st treatment with Easterna Aminofert /GreenzitS.P_{100} / Spinosad estimated at 0.63 larvae/10 bolls in comparison to the untreated check (1.47 larvae), in the 2007 season while the other sequences resulted in higher levels of infestation (ranging from 1.07 to 1.17 larvae/10 bolls), but still less than the untreated check.

B. Effects on the spiny bollworm

In the 2006 season it was noticed that the treatment of Easterna Aminofert / Greenzit S.P_{100} / Spinosad resulted in a significant reduction of the infestation rate (1.03 larvae/10 bolls), followed by the spray sequences of Easterna Aminofert / Greenzit S.P_{100} / Methoxyfenozide and Easterna Aminofert / Super Biovert / Spinosad (1.23 and 1.17 larvae/10 bolls, respectively) (**Table 3**). In contrast, the sequences of Easterna Aminofert / Super Biovert with Chlorpyrifos or Methoxyfenozide increased the infestation level significantly (1.53 and 1.57 larvae/10 bolls, respectively) compared with the untreated check. This can be explained by the fact that both Chlorpyrifos and Methoxyfenozide were not effective enough to decrease the infestation by bollworms. In fact, this apparent contradiction may be due to many factors such as the resistance and insensitivity of the insects themselves toward these compounds. Also, some interfering factors for pesticides inactivity include the nature of the sprayed surface as well as the age of insecticide deposits and environmental conditions such as humidity and temperature (Fletcher and Axtell 1993). In some pesticides and chemicals breakdown may also be expected on surfaces exposed to sunlight (Franko *et al.* 2005).

On the other hand, in the 2007growing season, the sequences of Easterna Aminofert / Greenzit S.P_{100} / Spinosad, Easterna Aminofert / Greenzit S.P_{100} / Chlorpyrifos and Easterna Aminofert / Super Biovert / Spinosad significantly decreased the infestation level of spiny bollworm, never exceeding 0.9 larvae/10 bolls in comparison to the untreated check of 1.97 larvae/10 bolls. In case of the sequences of Easterna Aminofert / Super Biovert with methoxyfenozide or chloropyrifos and Easterna Aminofert / Greenzit S.P_{100} / Methoxyfenozide, the highest infestation levels were 1.77, 1.37 and 1.30 larvae/10 bolls, respectively.

Effects on cotton yield

From **Table 4** it is obvious that there were non-significant differences among the treatment means for cotton yield; highest cotton yield (201.1%) was obtained from the Easterna Aminofert / Super Biovert / Spinosad treatment, higher than the untreated check. The other applied foliar sequences resulted in lower cotton yields but always higher than the untreated check, ranging between 8.1% (Easterna Aminofert / Super Biovert / Chlorpyrifos) and 63.3% (Easterna Aminofert / Greenzit S.P_{100} / Spinosad) in the 2006season. In the 2007growing season, in contrast, the Easterna Aminofert / Super Biovert / Spinosad treatment gave the highest increase in cotton yield, 214.2% more than the untreated check, followed by the foliar spray of Easterna Aminofert / Greenzit S.P_{100} / Spinosad (157.1% higher than the control). In all remaining applied foliar sequences, cotton yield was lower (ranging from 14.2 to 71.1%) than the untreated check.

Effects on urease and dehydrognase enzymes in soil

The interactions between the sequences and soil enzymes are presented in **Table 5**. The Easterna Aminofert / Greenzit S.P_{100} with Spinosad, Methoxyfenozide and Easterna Aminofert / Super Biovert with Spinosad and/or Methoxyfenozide sequences significantly decreased urease activity, 67.04, 61.07, 60.4 and 60.61%, respectively. Although the Easterna Aminofert / Super Biovert / chlorpyrifos sequence treatment had a slightly significant stimulative effect on urease activity at the end of the 1st spray a similar effect (80.8% increase in activity) was observed by the Easterna Aminofert / Greenzit S.P_{100} / Chlorpyrifos treatment in the 2nd spray.

Table 4 Effects of tested compounds on the cotton yield during the 2006 and 2007 growing seasons.

Treatments	2006		2007	
	Weight / feddan Kg (ken*.)	**% increase***	**Weight / feddan Kg (ken.)**	**% increase****
1. Easterna Aminofert / Greenzit SP_{100} / Spinosad	707.2 kg (4.4 ken.)	63.6	720 kg (4.5 ken.)	157.1
2. Easterna Aminofert / Greenzit SP_{100} / Methoxyfenozide	667.2 kg (4.2 ken.)	54.4	480 kg (3.04 ken.)	71.4
3. Easterna Aminofert / Greenzit SP_{100} / Chlorpyrifos	532.8 kg (3.3 ken.)	23.3	336 kg (2.1 ken.)	20
4. Easterna Aminofert / Super Biovert / Chlorpyrifos	467.2 kg (2.9 ken.)	8.1	320 kg (2.03 ken.)	14.2
5. Easterna Aminofert / Super Biovert / Spinosad	1300.8 kg (8.2ken.)	201.1	880 kg (5.5 ken.)	214.2
6. Easterna Aminofert / Super Biovert / Methoxyfenozide	667.2 kg (4.2 ken.)	54.4	448 kg (2.8 ken.)	60
Untreated check	432 kg (2.7 ken.)		280 kg (1.8 ken.)	

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

Table 5 Effects of tested compounds on the soil enzymes (urease and dehydrogenase).

Treatments	Urease enzyme			Dehydrogenase enzyme		
	1st spray	**2nd spray**	**3rd spray**	**1st spray**	**2nd spray**	**3rd spray**
1. Easterna Aminofert / Greenzit SP_{100} / Spinosad	67.04 b	72.5 b	74.31 b	45.30 a	27.04 a	27.73 cd
2. Easterna Aminofert / Greenzit SP_{100} / Methoxyfenozide	61.07 bc	49.72 c	75.31 b	46.80 a	33.68 b	36.33 bc
3. Easterna Aminofert / Greenzit SP_{100} / Chlorpyrifos	69.66 ab	80.80 a	63.14 bc	42.45 ab	55.84 a	46.36 ab
4. Easterna Aminofert / Super Biovert / Chlorpyrifos	82.55 a	72.21 ab	63.78 bc	45.38 a	42.37 ab	54.35 a
5. Easterna Aminofert / Super Biovert / Spinosad	60.40 bc	60.40 bc	65.44 bc	20.67 b	27.88 b	17.87 d
6. Easterna Aminofert / Super Biovert / Methoxyfenozide	60.61 bc	57.32 c	71.57 ab	34.60 ab	31.18 b	33.31 bc
Untreated check	78.18 a	73.65 a	73.65 a	72.52 a	72.58 a	72.58 a
F. cal	109.45	98.11	N.S	101.52	95.52	89.65
F. tab.	80.52	76.95		70.32	65.25	68.58
$L.S.D_{.05}$	51.50	12.86		22.16	15.16	12.86

Means followed by the same letter(s) are not significantly different at the 5% level

Also in the 2nd spray, the Easterna Aminofert / Greenzit $S.P_{100}$ / Methoxyfenozide, Easterna Aminofert / Super Biovert / Spinosad and Easterna Aminofert / Super Biovert / Methoxyfenozide sequences appeared to have a moderate effect (49.72, 60.4 and 57.32%, respectively). There were non-significant differences among the sequences following the 3rd spray.

DISCUSSION

To avoid unfavourable side effects of pesticides on beneficial insects, natural enemies and the environment, and to reduce outbreaks of cotton pests, an alternative approach for integrated pest management (IPM) was initiated recently to minimize the role of chemical pesticides. These results are in agreement with those of El-Naggar (2003) and Mesbah *et al*. (2004a, 2004b), who illustrated that the application of Batyhroid® with Greenzit $S.P_{100}$ lowered infestation by pink and spiny bollworms. Abdel-Rhman (2004) found that the highest reduction in cotton bollworm infestation was induced by Chlorpyrifos, Spinosad and Methoxyfenozid sequences.

Also, the obtained results are in agreement with those of Mesbah *et al*. (2000), who studied the effect of some fertilizers and foliar application of nutritive elements on the infestation of cotton with bollworms. Also, the findings of El-Nawawy *et al*. (1983), in which some foliar fertilizers such as Greenzit NPK and Byrthroid-Polytrin® and different insecticides as Malathion and Chlorpyrifos were used in sequences and mixtures as protectants of cotton bolls against pink bollworm. The joint action of these pesticide mixtures and foliar fertilizers gave the best results for the control of pink bollworm and increased the number of cotton bolls ripening and opening simultaneously. El-Naggar (1998), Mesbah *et al*. (2000) and Hussein *et al*. (2002) concluded that the application of double and triple sequence sprays of Byrthroid-Polytrin® with Greenzit $S.P_{100}$, Greenzit NPK and Polymex / ascorbic acid increased cotton yield, similar to our findings (**Table 4**). Abdel-Rhman (2004) and Amin and Gergis (2006) also found that the Spinosad, Chlorpyrifos, and Methoxyfenozide sequence increased cotton yield. Similar results were also obtained by El-Mallah and Emam (1998), El-Naggar (2003) and Mesbah *et al*. (2004a, 2004b) when they evaluated some foliars sprays and micro-elements and alternative chemical compounds in sequences in an IPM program on the control of pink and spiny cotton bollworms.

All sequences strongly inhibited dehydrognase activity at the end of each spray (1st, 2nd and 3rd; **Table 5**). Soil dehydrogenase enzyme activity is considered as an indicator of oxidative biological activity and soil respiration (Casida *et al*. 1964). After the 3rd spray with any treatment there was an insignificant effect on urease activity (**Table 5**). The effects and the regained activities of both these soil enzymes and microbes can be explained by the fact that many pesticides have significant effects on these microbes but they can recover rapidly (Widenfalk *et al*. 2008). Since enzyme activity reflects the activity of the existing microorganisms, it is thus expected from these results that the microbes can also be recovered. Diurak and Kazanici (2001) found that these deleterious effects on the activity of soil enzymes are not drastic but minor in nature.

On the other hand, it is known (Diurak and Kazanici 2001) that urea is used as a source of nitrogen that requires enzymatic hydrolysis to create nitrogen content to be available to plants. Urease catalyses hydrolysis of urea that could be absorbed by plant roots and probably causes a harmful effect on plant cells. Thus, a serious problem may arise when urea fertilizer is added to soil having low urease activity (Diurak and Kazanici 2001). The Easterna / Greenzit $S.P_{100}$ with Spinosad and Methoxyfenozide, Easterna / super Biovert with Spinosad and/or Methoxyfenozide sequences had a decreasing effect on urease activity (67.04, 61.07, 60.4 and 60.61%, respectively). However, with the Easterna / Super Biovert / chlorpyrifos sequence had a slight weak effect (82.55%) on urease activity at the end of the 1st spray. These findings illustrate that the highest decreasing effect was observed with the 5th treatment (20.67 ppm formazan) after the 1st application of the 1st, 2nd, 5th and 6th treatments giving 27.04, 33.68, 27.88 and 31.18 ppm formazan. On the other hand, after the 2nd application of the 1st and 5th treatment and after the 3rd application 27.73 and 17.87 ppm formazan resulted. This study indicates that dehydrogenase is more influenced by the sequences that revealed with urease enzyme.

According to the level to which cotton bollworms infestation in different sequences was reduced, it is evident that the Easterna Aminofert / Greenzit $S.P_{100}$ sequence with chloropyrifos and/or Spinosad in 2006 and the 1st treatment in 2007 resulted in the greatest reduction of pink and spiny bollworms. In the 2nd spray, treatment with the 3rd treatment stimulated urease activity (80.8%) while the 2nd, the 5th and the 6th treatment appeared to have a moderate effect (49.72, 60.4 and 57.32%, respectively). Moreover, the 3rd spray exhibited non-significant differences among the sequences. All sequences highly inhibited dehydrognase activity at the end of each spray (**Table 5**).

These findings illustrate that the highest decreasing effect here was related to Easterna / Super biovert / Spinosad (20.67 ppm formazan). Formazan dyes are artificial chromogenic substrates for dehydrogenases and reductases. Dehydrogenase activity in the soil sample was determined as a result of the reduction of 2,3,5- tripheneyltetrazolium chloride (TTC) to triphenyl formazan (TPF) which is extracted and measured spectrophotometrically (Casida *et al.* 1964). It was observed that high decreasing effect occurred after the 1st application of the 1st, 2nd, 5th and 6th treatment estimated at 27.04, 33.68, 27.88 and 31.18 ppm formazan, respectively. Also the decrease occurred after the 1st, 2nd and 5th treatment after the 3rd application (27.73 and 17.87 ppm formazan, respectively).

These results indicate that dehydrogenase is more influenced by the sequence than urease. In fact, the influence of on the activity of various pesticides was examined by Sannin and Gianfreda (2001), who studied the effect of four pesticides, e.g. glyphosate, paraquat, atrazine, and carbaryl, on the activities of invertase, urease and phosphatase. The addition of glyphosate and paraquat activated invertase and urease activities in several soils. Smaller increases were measured for urease. A general inhibitory effect (from 5 to 98%) was observed for phosphatase in the presence of glyphosate. The effects of atrazine and carbaryl on the three soil enzymes were evaluated against that exhibited by methanol, the solvent used for their solubilization. In almost all soils, atrazine further inhibited invertase activity with respect to the inhibitory effect shown by methanol. By contrast, consistent activation effects were measured for urease with methanol alone and/or methanol-pesticide mixtures.

According to the level of reduction of cotton bollworm infestation in different sequences, it is evident that the Easterna Aminofert / Greenzit S.P$_{100}$ sequence with chloropyrifos and/or Spinosad in 2006 and the Easterna / Greenzit S.P$_{100}$ / Spinosad sequence in 2007 resulted in the greatest reduction of pink and spiny bollworms. From these results we may conclude that Spinosad and Chlorpyriphos were more effective against pink and spiny bollworms when incorporated with the Easterna Aminofert / Greenzit S.P$_{100}$ sequence than the control and other compounds. This result is in line with that of Ravi *et al.* (1997) who concluded that Chlorpyriphos (0.3%) was effective against the 1st, 3rd and 5th instars larvae of pink bollworm. Allen *et al.* (2000) and Johnson *et al.* (2000) also concluded that Spinosad (0.025%) provided good control of American bollworm, *Helicoverpa armigera*. Spinosad generally kills insects through activation of the acetylcholine nervous system through nicotinic receptors. The mode of action is unique and incompletely understood. Continuous activation of motor neurons causes insects to die of exhaustion. There may be some effects on the neurotransmitter gamma-aminobutyric acid (GABA) and other nervous systems (Thompson *et al*. 2000). Moreover, this unique mode of action of Spinosad, coupled with a high degree of activity especially on pink and spiny bollworms, low toxicity to non-target organisms (including many beneficial arthropods), and resistance management properties make Spinosad an excellent new tool for IPM (Khan *et al*. 2007).

On the other hand, the use of foliar nutrients postulates a plant nutritional state with optimal levels and proportions among minerals that enhances plant growth and suppresses herbivore performance (Beanland et al. 2003). Therefore, the applied pesticide sequences and foliar nutrients help greatly in this respect. Besides accessing nutrients, for current intake as well as residual, different nutrients also provide growth-promoting factors to plants and some plant growth promoters and other foliar nutrients have successfully facilitated composting and effective recycling of solid wastes (Atiyeh *et al.* 2000). By controlling soil-borne diseases and other pests and improving soil health and properties these nutrients and PGP help not only in this respect, but also result in higher yield rates as was observed in this study. Taking into consideration the severe damage caused by bollworms (the most serious cotton pests in Egypt), these results are likely to be commercially promising under Egyptian conditions in the long run once information becomes available to producers and farmers through experience and communication.

CONCLUSIONS

The results of this study indicate that the foliar treatment of Easterna Aminofert / Greenzit S.P$_{100}$ / Spinosad decreased the incidence of spiny bollworm infestation in the 2006 cotton season. The Easterna Aminofert / Greenzit S.P$_{100}$ with Spinosad and/or Chlorpyrifos also effectively reduced the level of spiny bollworm infestation in the 2007 season. The lowest infestation value of pink bollworm was induced by Easterna Aminofert / Greenzit S.P$_{100}$ with Chlorpyrifos and Spinosad in the 2006 season and by the Easterna Aminofert / Greenzit S.P$_{100}$ / Spinosad sequence in the 2007 season. The application of Easterna Aminofert followed by Super Biovert and Spinosad in this order gave the greatest cotton yield compared to the untreated check in both 2006 and 2007 cotton seasons. Easterna Aminofert / Greenzit S.P$_{100}$ / Spinosad only resulted in high cotton yield in 2007. Soil dehydrogenase was more influenced by the evaluated sequences than urease.

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