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# Non-traditional approaches against spiny bollworm *Earias insulana* (Lepidoptera: Noctuidae) infesting cotton in Egypt

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**Abstract:**

The spiny bollworm *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) is a major insect pest of cotton in Egypt. The present field study was carried out to evaluate the influence of nine treatments against *E. insulana* during two successive seasons 2018 and 2019. Agricultural practices including the 1$^{st}$ and 2$^{nd}$ treatments; plowing, irrigation and fertilization that showed a low percent of infestation reduction, while 3$^{rd}$ treatment including the bio-rational insecticides of (Azdirachtin, emamectin benzoate, spinosad) showed a reasonable infestation reduction, moreover, the 4$^{th}$ treatment was the release of the predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) alone achieved good results compared to the previous treatments, while, the 5$^{th}$ treatment was *Bacillus thuringiensis* and the 6$^{th}$ treatment was *Beauveria bassiana*, the 5$^{th}$ and 6$^{th}$ treatments were the least in infestation reduction when applied alone. However, the 7$^{th}$, 8$^{th}$ and 9$^{th}$ treatments represent the three different combined treatments resulted in very good infestation reduction and were more effective during the two successive seasons of study 2018 and 2019. Combined treatments increased the infestation reduction and were of great value and should be applied to the promising ones only. This research paper may greatly improve the current knowledge and practices for sustainable development, ecology and environmental protections.

## Introduction

The genus *Earias* has an extremely wide range through cotton-growing countries and is found to be distributed among most African countries, the Mediterranean basin, India, China, and Southeastern Asia (Reed, 1994). Due to its severity of infestation and type of damage, spiny bollworm *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) was recorded as a major pest and caused most serious cotton damage. Larvae attack and damage squares, flowers and cotton bolls; consuming seeds, destroying them with accumulation of feces which serve as a suitable media for secondary pests and fungus, larvae can destroy all the cotton bolls in the field resulting in yield losses (Van Hamburg and Guest, 1997; Hanumantharaya *et al.*, 2008; Ahmed *et al*., 2012; Pappas *et al.*, 2011 and Bennett, 2015). There are many ways to reduce *E. insulana* infestation like cultivation of the resistance cotton varieties (Moustafa *et al*., 2015).

The green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) is an important natural predator of many insect species attacking different crops. Chrysopids are generalist predators that recognized to be a voracious feeder on arthropod pests such as; aphids, whiteflies, eggs and larvae of cotton bollworms (Senior and McEwen, 2001; Atlihan *et al*., 2004; Zia *et al.*, 2008 and Soomro *et al.*, 2020). In addition, Bhatti *et al.* (2007) reported that *C. carnea*, cause reasonable reduction in bollworms population.

The importance of bio-insecticides as alternative management methods is environmentally friendly and can help in natural balance. Such alternative bio-insectidies as, emamectin benzoate is a semisynthetic avermectin insecticide derived from the fermentation product avermectin B1 and it works as a chloride channel activator by binding gamma aminobutyric acid (GABA) receptor and glutamate-gated chloride channels disrupting nerve signals within arthropods (Grant, 2002). Spinosad is derived from a naturally occurring soil actinomycete bacterium, *Saccharopolyspora spinosa* (Thompson *et al*., 1997) binding target sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system then cause disruption of acetylcholine neurotransmission (Qiao *et al*., 2007).

*Bacillus thuringienesis* (*Bt.*)depends on endotoxin activity through the larval midgut which in turn affects the permeability of the epithelial cells and hence causing intoxication of the larval hemolymph. Entomopathogenic fungus spores contact with the insect host body then germinate, penetrate the cuticle, and grow inside, killing the insect within a few days. Several species of entomopathogenic fungi produced commercially and used as biological control agents against many insect pests in many parts of the world as *Beauveria bassiana* isolates (Sevim *et al*., 2015). The spores are sprayed on affected crops as an emulsified suspension or wettable powder. And, bio-insecticides like spinosad, emamectin benzoate and entomopathogeic fungi have less harmful effect on *C. carnea* (Moustafa, 2016a and Moustafa *et al*., 2019). Also, application of plants extracts considered one of the best control measures because of their less toxicity against non-target organisms and their biodegradability (Singh *et al*., 2001). Yousef and Moustafa (2013) found that *Melia azedarach* oil proved promising results against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) . Among the plants with insecticidal activities, *Azadirachta indica* is the most promising, its natural compound, azadirachtin has been known to possess insecticidal properties for several years and it is active against nearly 550 insect species (Anuradha and Annadurai, 2008) and acts as antifeedant and repellant (Chaudhary *et al*., 2017).

In this study, field application by using non-traditional and environmentally safe treatments such as azadirachtin, emamectin benzoate and spinosad, as well as, introducing the predator *C. carnea* as a biological control agent were used for spiny bollworm *E. insulana* management.

## Materials and methods

Trials were conducted at Qaha experimental station, Qalyoubia Governorate, an area about 1400 m$^2$

was cultivated with Giza 86 cotton variety at March 31st, 2018 and March 13th, 2019 seasons without chemical receive to evaluate the effect of all treatments on the population density of spiny bollworm infesting cotton plants. The trial was designed as randomized complete blocks, nine plots were designed for the treatments' application and one plot was left without treatments as a control (check), by three replicates for each plot.

**1. Agricultural practices:**

Prior to cultivation the soil was plowed using farming plow machine. Seeds are typically planted an inch below the soil surface, plowing give those seeds the best chance for growing and germination. Plowing breaks up the blocky structure of the soil which can aid in drainage and root growth. As cotton plants suffer drought during spring and summer months, therefore, plants of this treatment were irrigated during April, May, July and August each season, the commercial fertilizer (NPK), nitrogen: phosphorus: potassium at the rate of 20:10:10, these agricultural treatments were done free of insecticides application. Fertilization treatment was applied as follow; nitrogen at April, while, phosphate was applied after plowing and potassium at June.

**2. Data of commercial formulation insecticides used:**

**2.1. Bio-rational insecticides treatments:**

Recommended concentration of the bio-rational insecticides used as follow; Oikos 3.2% EC (Azadirachtin) obtained from Lutus company by rate of application 100cm/100Litre water, exellent 1.9% EC (Emamectin benzoate) obtained from Kafr El-Ziat for Pesticides and Chemicals by rate of application 300cm/feddan, and spintor 24% SC (Spinosad) obtained from Daw Chemicals Company by rate of application 50cm/feddan were locally sprayed alternatively two weeks interval between sprays.

**2.2. Microbiological treatments:**

**2.2.1. Bacterial treatment:**

Zentari 54% DF (*B. thuringiensis*, subsp. Aizawai, Strain ABTS-1857) obtained from Shoura for Chemicals Company by rate of application 200gm/feddan was locally sprayed on the stem, branches, twigs and the whole vegetative system.

**2.2.2. Fungal treatment:**

Bio-Power 1.15% WP (*Beauveria bassiana*, 1 x 108 spores i.e., CFU/g) obtained from Gaara Company at the rate of 1.5 kg/feddan were sprayed on stem, branches, twigs and the whole vegetative system.

**3.** Spraying for each treatment was conducted three times by two weeks interval between each spray. Treatment spraying was practiced by a knapsack sprayer Cifarili and mainly directed towards the vegetative system.

**4. Release of *Chrysoperla carnea*:**

The predator was obtained from the Center of Bio-Organic Agricultural Services (CBAS) in Aswan. Release of *C. carnea* 2nd instar larvae in the ratio of 250 larvae/200 $m^2$, they were simply sparse over the pest infested cotton plants was implemented three times at fourteen days intervals.

**5. Combined treatments:**

Three combinations of simultaneous treatments were applied. These include bio-rational insecticides spray and predator release, the second and third combined treatments were bio-rational insecticides spray, predator release and microbiological treatments. Check plants were left untreated for control. Insecticides were sprayed and predator release, with an interval of fourteen days. Dates of bio-rational insecticides and microbiological treatments application as following; the 1st, 2nd and 3rd spray were applied at June 25th, July 9th and July 25th during season 2018, whereas, they were July

1st, July 15th and July 30th 2019. Moreover, the three predator releases dates were as follow; July 2nd, July 16th and July 30th during season 2018, while, they were July 8th, July 22nd and August 6th during season 2019. However, dates of spray the three combined treatments were as following; the 1st combined treatment sprayed at June 25th, July 9th and July 25th during season 2018 and July 1st, July 15th and July 30th 2019 and predator released between them at July 2nd, July 16th and July 30th season 2018, while, they were July 8th, July 22nd and August 6th during season 2019. The 2nd and 3rd combined treatments sprayed at June 25th, July 9th, July 25th and August 1st during season 2018 and July1st, July15th, July 30th and August 6th during season 2019 and predator released between them.

To evaluate the effect of the nine treatments against spiny bollworm, samples of 50 bolls/plot were randomly picked weekly before and after each treatment until harvest. Check plants were left untreated for control. The collected bolls were carefully dissected, and number of exit holes was recorded. Percentage of larval infestation reduction in green boll was estimated according to **Henderson and Tilton (1955).**

**6. Statistical analysis:**

The recorded data were statistically analyzed with CoStat program software [one-way analysis of variance (ANOVA) (P <0.05%) Duncan's multiple range test of means (Duncan, 1955)].

**Results and discussion**

Data given in Tables (1 and 2) showed that the infestation reduction of nine treatments during the two successive seasons 2018 and 2019 as following:

**1. Agriculture treatments:**

**1.1. Effect of plowing treatment alone:**

The infestation reduction with *E. insulana* due to this treatment was low, it was 2.0, 3.5, and 2.0% during 2018 (Table 1) and was 4.5, 3.0 and 2.5% during 2019 for the infested squares, flowers and bolls, respectively (Table 2).

**1.2. Effect of irrigation and fertilization treatment:**

Slight degree of *E. insulana* infestation reduction was achieved when irrigation and fertilization treatments were applied together. The reduction percentages of the pest infestation reached 5.0, 8.0 and 6.5% during 2018 season and 9.5, 7.0 and 6.6% for squares, flowers and bolls during 2019 season, respectively.

**2. Effect of bio-rational insecticides spray:**

**2.1. Alternative spray of azadirachtin, emamectin benzoate and spinosad:**

Remarkable degree *E. insulana* infestation reduction was noticed (Table 1) when the alternative three applications of azadirachtin, emamectin benzoate and spinosad spray were applied. Insignificant infestation reduction percentages of *E. insulana* were 71.75 and 76.77% for squares and flowers, respectively and but significant reduction was 78.72% for bolls during 2018 season. During 2019 season, application of three alternative bio-rational insecticides sprays; azadirachtin, emamectin benzoate and spinosad resulted in significant grand infestation reduction by 73.98 and 81.32% for squares and bolls, respectively and significant reduction in flowers by78.07% ( Table 2).

**Table (1): Effect of different treatments on reduction percentages of infestation by spiny bollworm *Earias insulana* larvae during season 2018.**

| Treatments | 2018 season | | | | | |
|---|---|---|---|---|---|---|
| | Squares | | Flowers | | Bolls | |
| | Mean No. of infested squares | % Reduction | Mean No. of infested flowers | % Reduction | Mean No. of infested bolls | % Reduction |
| **1.Agricultural treatments:**<br>**1.1. Plowing treatment** | 19.8 | 2.0%[c] | 22.9 | 3.5%[b] | 17.2 | 2.0%[c] |
| **1.2. Irrigation and fertilization treatment:** | 16 | 5.0%[c] | 17.1 | 8.0%[b] | 21.05 | 6.5%[c] |
| **2.Bio-rational insecticides spray:**<br>**2.1. Alternative spray of azadirachtin, emamectin benzoate and spinosad.** | 7.9 | 71.75%[b] | 6.5 | 76.77%[a] | 8.33 | 78.72[%b] |
| **3. Predator release:**<br>**3.1. *Chrysoperla carnea* release.** | 2.4 | 66.48%[b] | 3.2 | 72.92%[a] | 10.17 | 75.77%[b] |
| **4. Microbiological treatment:**<br>**4.1. Bacterial treatment: *Bacillus thuringeinsis*** | 7.3 | 11.70%[c] | 6.5 | 9.18%[b] | 14.5 | 12.35%[c] |
| **4.2. Fungal treatment: *Beauveria bassiana*** | 12 | 8.60%[c] | 10.3 | 10.50%[b] | 8.2 | 11.60%[c] |
| **5.Combined treatments**<br>**5.1. Treatment 2.1. +3.1.** | 18 | 86.55%[a] | 16.5 | 79.71%[a] | 22.5 | 81.00%[a] |
| **5.2. Treatment 2.1. +3.1. +4.1.** | 3.9 | 88.20%[a] | 3.5 | 81.50%[a] | 3.2 | 83.63%[a] |
| **5.3. Treatment 2.1. +3.1. +4.2.** | 5.4 | 87.00%[a] | 4.1 | 79.90%[a] | 6.3 | 84.60%[a] |
| **Untreated (Check)** | 24.83 | ---- | 27.08 | ---- | 31.58 | ---- |
| **LSD** | | 14.18 | | 13.62 | | 13.69 |
| **F** | | 76.81 | | 65.16 | | 36.56 |
| **P-value** | | .0000*** | | .0000*** | | .0000*** |

**Values within the same column followed by the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).**

## 3. Effect of predator release:

### 3.1. Release of *Chrysoperla carnea*:

Releasing three times of *C. carnea* 2nd instar larvae showed insignificant reduction in the squares and flowers by 66.48 and 72.92% but significant reduction in bolls infestation caused by *E. insulana* by 75.77%, respectively, during season 2018 (Table 1). *C. carnea* 2nd instar larvae release showed significant general infestation reduction for the three release in flowers by 82.76% but insignificant general reduction in squares and bolls reached 70.88 and 78.28%, respectively during season 2019 (Table 2).

**Table (2): Effect of different treatments on infestation reduction percentages of by spiny bollworm *Earias insulana* larvae during season 2019.**

| Treatments | 2019 season | | | | | |
|---|---|---|---|---|---|---|
| | Squares | | Flowers | | Bolls | |
| | Mean No. of infested squares | % Reduction | Mean No. of infested flowers | % Reduction | Mean No. of infested bolls | % Reduction |
| **1. Agricultural treatments 1.1. Plowing treatment** | 6 | 4.5%[c] | 16 | 3.0%[c] | 21 | 2.5%[d] |
| **1.2. Irrigation and fertilization treatment** | 7.9 | 9.5%[c] | 18 | 7.0%[c] | 17 | 6.6%[d] |
| **2. Bio-rational insecticides spray: 2.1. Alternative spray of azadirachtin, emamectin benzoate and spinosad** | 3.3 | 73.98%[b] | 4.2 | 78.07%[b] | 5.67 | 81.32%[b] |
| **3.Predator release 3.1. *Chrysoperla carnea* release** | 2 | 70.88%[b] | 2.5 | 82.76%[a] | 6.75 | 78.28%[b] |
| **4. Microbiological treatment 4.1. Bacterial treatment: *Bacillus thuringeinsis*** | 8 | 9.43%[c] | 11 | 8.43%[c] | 10 | 13.33%[c] |
| **4.2. Fungal treatment: *Beauveria bassiana*** | 11 | 10.14%[c] | 8.5 | 13.55%[c] | 17.5 | 14.10%[c] |
| **5.Combined treatments 5.1. Treatment 2.1. +3.1.** | 7 | 88.06%[a] | 15.5 | 82.73%[a] | 19 | 86.00%[a] |
| **5.2. Treatment 2.1. +3.1. +4.1.** | 2.9 | 87.90%[a] | 3 | 88.00%[a] | 6.2 | 90.54%[a] |
| **5.3. Treatment 2.1. +3.1. +4.2.** | 1.9 | 81.00%[ab] | 1.1 | 84.60%[a] | 2.1 | 87.00%[a] |
| **Untreated (Check)** | 11.3 | --- | 21.97 | --- | 26.75 | --- |
| **LSD** | | 11.67 | | 15.17 | | 11.15 |
| **F** | | 95.70 | | 40.56 | | 111.39 |
| **P-value** | | .0000*** | | .0000*** | | .0000*** |

**Values within the same column followed by the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).**

### 4. Effect of microbiological treatments:

### 4.1. Effect of *Bacillus thuringiensis* treatment:

Results in Table (1) showed that after three sprays of *B. thuringiensis* treatment; non-significant general infestation reduction percentage of *E. insulana* was as following; 11.70, 9.18 and 12.35% in squares, flowers and bolls, respectively, during season 2018. Results in Table (2) showed non-significant general infestation reduction caused by *E. insulana* after the three sprays of bacterial treatment was as following; 9.43, 8.43 and 13.33% in squares, flowers and bolls, respectively, during season 2019.

### 4.2. Effect of *Beauveria bassiana* treatment:

Results in Table (1) showed that after three sprays of *B. bassiana*; non-significant general reduction percentages of *E. insulana* infestation was 8.60, 10.50 and 11.6% in squares, flowers and bolls, respectively, during season 2018. Moreover, results in Table (2) showed that the general reduction of the three sprays of fungal treatment was 10.14, 13.55 and 14.10% in squares, flowers and bolls, respectively, during season 2019.

### 5. Effect of combined treatments:

### 5.1. Effect of alternative spray of azadirachtin, emamectin benzoate and spinosad and *Chrysoperla carnea* release:

This treatment showed good results by 86.55, 79.71, 81.00% infestation reduction on squares, flowers and bolls respectively non-significantly during 2018 season (Table 1), on the other hand, the same combined treatments gave 88.06, 82.73 and 86.00% infestation reduction on squares, flowers and bolls, respectively, during 2019 season (Table 2).

**5.2. Effect of alternative spray of azadirachtin, emamectin benzoate and spinosad and *Chrysoperla carnea* release and bacterial treatment:**

Satisfied results were obtained from this treatment during 2018 season by 88.2, 81.50 and 83.63% infestation reduction non-significantly on squares, flowers and bolls, respectively (Table 1**),** while, it was 87.9, 88.00 and 90.54% infestation reduction on squares, flowers and bolls, respectively, during 2019 season (Table 2).

**5.3. Effect of alternative spray of azadirachtin, emamectin benzoate and spinosad and *Chrysoperla carnea* release and fungal treatment:**

This treatment showed very good results 87.00, 79.90 and 84.60% infestation reduction non-significant during 2018 season (Table 1) and 81.00, 84.60 and 87.00% on squares, flowers and bolls, respectively, during 2019 season (Table 2). Results of this study revealed that combined treatments were more effective against *E. insulana* infestation during the two successive years of study. Such observations were supported by Mirmoayedi *et al*. (2010), they applied botanical and bio-control methods to manage bollworms, and they evaluated the more efficient insecticide between three bio-insecticides in a chemical control of spiny bollworm. Spinosad had the lowest number of damaged bolls and had the minimum number of blind damaged bolls, followed by neem-azal and *Bt*. Similarly, Moustafa (2016 b) showed that *E. insulana* larvae was more susceptible to the toxicity of *M. azedarach* than *P. gossypiella* larvae after 2, 5 and 7-days post laboratory treatments. Moreover, AgNPs from *O. marjorana* plant leaf extract showed promising toxicity against *E. insulana* (Al Shater *et al.*, 2020). And, Nboyine *et al.* (2013) assessed field efficacy of neem-based bio-pesticides, obtained results showed that neem reduced the abundance of bollworms. Alkaloids and hydrocarbon of the pepper oil substances exhibited latent effect against the newly hatched larvae of spiny bollworm (El –Mesallamy *et al*.,2015). Venugopal *et al*. (2017) showed somehow similar results when they applied *Beauveria bassiana* on cotton crop (*Gossypium hirsutum* L.) against pink bollworm *P. gossypiella* they found that *B. bassiana* may be useful in devising proper integrated pest management strategy against pink bollworm.

Regarding, predator release, many reports agreed with the obtained data such as Salman *et al.* (2014) found that biological control treatments of releasing *C. carnea* decreased significantly the population of spiny bollworm *E. insulana*. Concerning combined treatments Mansoor *et al.* (2007) showed that integration of bio-control agents such as *C. carnea* with insecticides was effective as chemical control using recommended insecticides against spotted bollworms. Also, Hanumantharaya *et al.* (2008) used combined treatments of fertilizers, bio-control agent and botanicals to manage the insect pests on cotton, two sprays of neem seed kernel extract (5%) on cotton and release of *C. carnea* reduced *Helicoverpa armigera* (Hub.) eggs and larval population and increased the seed cotton yield. Similarly, Sree *et al*. (2019) found that five combined treatments of fertilization and bio-control agents cause high reduction in insect population of okra. Combined treatments based on azadirachtin and *B. thuringiensis* or azadirachtin and *B. bassiana* improved the efficacy of controlling *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Jallow *et al*., 2019).

It could be concluded that, from the foregoing results that combined treatments were more effective against *E. insulana* during the two successive years of study than any treatment alone. It was noticed that combined treatments increased the infestation reduction and were of great value and should be applied to the promising ones only. Although microbiological treatments were mostly ineffective during 2018 and 2019, respectively. Statistical analysis and grouping of nine treatments applied for two successive seasons 2018 and 2019, clarified that there was significance difference between treatments.

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