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Original Article

Efficacy of *Trichogramma Evanescens* (Hymenoptera: Trichogrammatidae), Six Pesticides, and Their Combinations to Control *Pectinophora Gossypiella* (Lepidoptera: Gelechiidae) in Egyptian Cotton Fields

Seham Mansour Ismail *

Insect Population Toxicology Department, Central Agricultural Pesticides Laboratory, Agriculture Research Center, 12618, Giza, Egypt

*Corresponding Author E-mail: dr.sehammansour1@gmail.com

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Abstract

Background: Pink bollworm, *Pectinophora gossypiella* Saunders (Lepidoptera: Gelechiidae) is one of the most economically devastating pests of cotton globally. Chemical control is the first line of defense, but has become ineffective in reducing yield losses caused by this pest. New management strategies against pink bollworm is urgently needed.

Methods: Field evaluations were conducted using the six pesticides were tested in two cropping seasons at a single dose, corresponding to their maximum recommended field rate of cotton in Egypt either with or without the egg parasitoid *Trichogramma evanescens*, TE (Hymenoptera: Trichogrammatidae). Treatments were applied to plots of cotton in 27th and 20th of July, 2021 and 2022, respectively, against pink bollworm, and efficacy was calculated based on the plant damage caused by the larvae through the yield.

Results: The highest reduction in pink bollworm larval numbers along with the least boll damage were found in azadirachtin, chlorantraniliprole, or hexaflumuron + *T. evanescens* (TE) plots. During both years, it was observed that plots treated with biological control agent and some pesticides led to a significant increase in cotton yield. The parasitism rates were 28.88, 24.19, 23.66, and 20.13% in the plots treated with azadirachtin, hexaflumuron, chlorantraniprol, and spinosad and only 2.80 and 1.57% in the plots treated with lambda-cyhalothrin and profenofos versus 33.62% in the control plot, there was difference significantly among treatments ($p \leq 0.05$). These findings underscore the potential use of TE combined with pesticides (mainly azadirachtin) to control pink bollworm larvae.

Conclusion: The promising results were achieved when combined TE with azadirachtin, the combined treatment significantly decreased the population of the pink bollworm and crop damage.

Keywords: Biological control, Chemical control, Cotton, Egg parasitoids, Parasitism, Selectivity

Introduction

One of the most significant commercial crops, cotton (*Gossypium* spp.), is connected with over 130 distinct species of arthropods [1]. One of these species is the pink bollworm, *Pectinophora gossypiella* which is considered an important pest of cotton crops a ranking as the most significant lepidopteran pest in practically all cotton-growing nations [2]. The larval stage of *P. gossypiella* which mostly attacks cotton, typically enters the boll and causes high yield losses of between 2.8-61.9% in seed, 2.1-47.10% in oil content, and 10.70-59.20% in normal boll opening [2, 3]. Chemical control is the first line of defense to control outbreaks of this pest and is still the main method used by farmers as up to 60% of all commercial insecticides are used in cotton [4, 5]. However, the extensive usage of harmful chemicals has negative effects on both the environment and human health. In addition, broad spectrum pesticides often have a much greater impact on natural enemies than on the target pest, which can lead to pest resurgence and the development of pest resistance [6]. To effectively control the target pests while having little to no effect on non-target arthropods like pathogens, parasitoids, and predators, new pest management strategies combining chemical and biological agents are urgently needed [7]. Since the use of selective pesticides is essential in the field, there is an ongoing need for detailed information on how they affect non-target arthropods for their protection [8].

In Egypt, *P. gossypiella* management has mostly focused on chemical control while this pest is attacked by a wide diversity of natural enemies, including parasitoids. Therefore, selective pesticides must be used to control this pest and maintain the natural enemies in the agroecosystem [9]. The genus Trichogramma shows different species responsible for natural control of several insects-pest, mainly lepidopterous [10, 11]. Hence, the study of side effects of pesticides is crucial to enhance the combined effect of chemical control and biological control tools. This work was done to examine the impact of six pesticides at the highest recommended dose rate in Egyptian cotton fields for the control of *P. gossypiella*, either with or without the egg parasitoid *Trichogramma evanescens* (TE) during two successive seasons. To gather information about these pesticides to elucidate fully their compatibility with TE to use in IPM programs.

Materials and Methods

Parasitoid Culture

The parasitoid of *Trichogramma evanescens* (TE, Tricho®) was purchased from International Co. for Biological Agricultural. The parasitoid was reared on eggs (<2 d old) of the cereal moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) as a factitious laboratory host. The parasitized eggs of the host were glued on white paper cards (15×10 cm) and exposed to parasitoid adults for 24 h to avoid super-parasitism, and then the cards were removed. The parasitoid was released in the experimental plots inside thick paper envelopes (5×8 cm) to protect them from predators' attack and unfavorable weather conditions. Each paper envelope was hung on the cotton plant approximately 50 cm above the ground. The distance between the release points was 14 m and started 7 m from apart from the edges of the field. The release of parasitoid TE was just before sunset. Rate of releases (24 cards about 1500-2000 parasitoids/card/fed., with a total of 40,000-48,000 parasitoids/fed.).

Tested Pesticides

In these experiments, six commercial formulations of pesticides from six chemical classes were used. Pesticides were applied at the maximum recommended field rate in cotton by the Egyptian Ministry of Agriculture to control of *Pectinophora gossypiella* (Table 1).

Table 1 Formulations and concentrations of the pesticides used in this study

Common name	Trade name	Manufacturer	Field rate (mL/fed.)
Spinosad SC 24%	Tracer®	Dow Agro Science	50
Profenofos EC 72%	Adwuprof®	Bayer Com.	750
Hexaflumuron EC 10%	Consult®	Dow Agro Science	200
Lambda-cyhalothrin EC 5%	Pulsar®	Samtrade Co.	375
Azadirachtin EC 0.15%	Achook®	Egyptian Agricultural development Co.	250 cm^3
Chlorantraniliprole SC 18.4%	Coragen®	Du Pont	47.50 g

Experimental Design and Timing of Application

The experiment was conducted for two successive seasons, summer 2021 and 2022, in cotton fields at Aga District, El-Dakahlia Governorate, (31° 6′ 16.5588″ N, 31° 22′ 53.4828″ E), Egypt. The cotton variety Giza70, sown on 25th and 16th of March at 105 × 60 cm spacing during both the seasons 2021 and 2022, respectively. All recommended agronomic practices were followed during the experiment were followed to properly manage the cotton crop. The area was divided into 14 plots (500 m^2) and wide ridges (1 m). Each plot was separated from the adjacent one by at least 100 m (as a barrier) to avoid interference between treatments. The trade names and recommended application rates for pesticides against *P. gossypiella* are provided in Table 1. All pesticides were sprayed in combination with a surfactant. Low volume knapsack sprayer (CP3) of 200 L/fed was used. Randomized complete block design (RCBD) was adopted for the trial with four treatments including untreated control which were replicated thrice. Four treatments were applied on each parcel constituting such complete blocks: releasing *T. evanescens* (TE) alone, applying pesticide alone, applying both together (TE + pesticide), and a control without any application or release. Pesticide application only on 27th and 20th of July, 2021 and 2022, respectively, when the percent of *P. gossypiella* infestation exceeded 3%. TE alone was released after the formation of the first fruiting branch on cotton plants around 15th of June in both seasons [12]. Applying both together where TE was released after 1, 3 and 6 days of pesticides application. In all experiments water was used as the control treatment.

Evaluation

The data was recorded to measure the infestation of pink bollworm larvae in each season through destructive sampling of green bolls. 100 green bolls were randomly selected weekly (11 weeks; July-September) from each treatment. Samples were examined externally before dissection and internal inspection and data were recorded on the same day of collection from all the treatments. Infestation records were based on the existence of injury symptoms regardless the larvae presence. Yield were further assessed at the time of harvesting in both seasons. The infestation rate was calculated according to the following formula:

$$Infestation\ rate = {}^{NI}/_{NT} \times 100$$

Where, NI: the number of infested bolls and NT: the total number of collecting bolls. To determine the parasitism percentage, the release cards were collected from each experimental plot, parasitized eggs were recorded under a binocular microscope. The persistence rate was evaluated according to each insecticide's IOBC/WPRS working group [13]. The parasitism rate was calculated as follows:

Parasitism rate = (average number of parasitized eggs of TE in the pesticide treatment/average number of eggs in the control treatment plot) x 100

Data Analysis

One-way analysis of variance (ANOVA) was used to statistically examine the data that were collected. Tukey multiple range test ($p \leq 0.05$) was used to identify differences between the treatments.

Results

3.1. Assessment of field infestation by Pectinophora gossypiella

The efficacy of six pesticides from different groups on *Pectinophora gossypiella* either with or without the parasite released *Trichogramma evanescens* (TE) was evaluated in field trials in two consecutive seasons (Figure 1). A single application of all tested pesticides resulted in a significant decrease in infection with pink bollworm when compared to use of TE only. Importantly, the infection rate of pink bollworm in pesticide-treated plots with TE was significantly lower than in pesticide-only treated plots. The mean infestation rate during the season 2020 was 2.6, 3.2, 3.9, 4.8, 5.5, 6.4, and 7.5% after treatment with azadirachtin, hexaflumuron, chlorantraniliprole, spinosad, lambda-cyhalothrin, profenofos, and released TE, respectively, while the control plots showed the highest overall mean of infestation (11.3%). In season 2021, the mean infestation rate was 4.0, 4.9, 5.5, 6.8, 7.7. 9.0, and 10.8%, respectively, compared with control plots recorded of 15.5%. After released TE, the mean infestation rate during the season 2020 recorded 1.4, 2.8, 3.6, 4.7, 5.4, and 6.3% for azadirachtin, hexaflumuron, chlorantraniliprole, spinosad, lambda-cyhalothrin, and profenofos, respectively, also, during the season 2021 recorded 2.2, 4.6, 5.2, 6.7, 7.6, and 8.9%, respectively.

Parasitism Rate

The data in Table 2 indicate that there are significant differences in the parasitism rates between the control and the treated plots during the two seasons. The highest parasitism rates were with azadirachtin-treated plots at all evaluation periods. The first release was conducted after 1 day of spraying pesticides, the parasitism rate was significantly higher in the control plots with 33.62% compared to 24.19, 23.66, 20.13, 2.80, and 1.57% in plots treated with hexaflumuron, chlorantraniliprole, spinosad, lambda-cyhalothrin, and profenofos, respectively. The parasitism rates registered in treated plots with hexaflumuron, chlorantraniliprole, profenofos, lambda-cyhalothrin, and spinosad were 18.80, 17.58, 12.76, 8.28,

and 6.24%, respectively for the second release (after three days from spraying), compared to 32.21% in control plots. At the third release (after five days from spraying), the parasitism rates registered in treated plots were 21.40, 21.06, 27.17, 17.72, and 4.00%, respectively, compared to 35.91% in control plots.
There were significant differences in parasitism rates among the treatments at all evaluation periods (Table 2). An ANOVA on the parasitism rates indicated a significant effect of pesticide and day of testing (pesticide treatment; $F = 28.78$; df = 7, 144; $p < 0.001$: day; $F = 12.55$, df = 3, 144; $p < 0.001$), and there was a significant interaction among these factors ($F = 6.35$; df = 21, 144; $p < 0.001$). Profenofos-treated plots had the lowest parasitism (IOBC class 3), followed by lambda-cyhalothrin and spinosad whereas azadirachtin (IOBC Class 1) did not differ from the negative control plots. Hexaflumuron and chlorantraniliprole (IOBC class 2) differ slightly from the negative control plots.

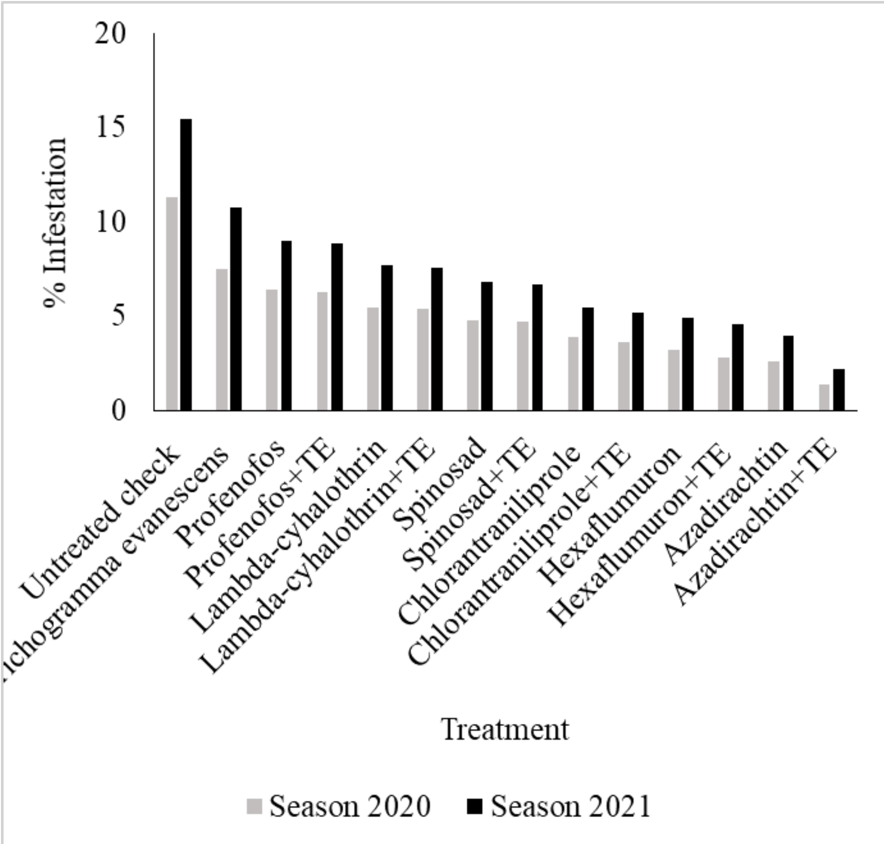

Figure 1 Infestation percentage of Pectinophora gossypiella larvae after treatment with six pesticides at the highest recommended dose rate in Egyptian cotton fields during two successive seasons, either with or without the egg parasitoid Trichogramma evanescens (TE)

At each evaluation period, the pesticides were categorized into four IOBC (International Organization for Biological Control) classes based on their reduction (R) in beneficial capacity compared to the negative control. Means followed by the same letter in the column do not differ, within each time interval from spraying, by Tukey's test ($p = 0.05$). *Class 1, harmless ($R < 30\%$); Class 2, slightly harmful ($30\% \leq R < 80\%$); Class 3, moderately harmful ($80\% \leq R \leq 99\%$); and Class 4, harmful ($R > 99\%$).

Average parasitism rate (± SE) of egg parasitoids Trichogramma evanescens (TE) during seasons 2020 and 2021 after applying six pesticides

Treatment	Days postspraying					
	1 d	R (IOBC class)*	3 d	R (IOBC class)*	6 d	R (IOBC class)*
Spinosad	20.13 (1.92)^{d}	40.12 (2)	6.24 (1.53)^{f}	80.63 (3)	4.00 (1.51)^{f}	88.86 (3)
Profenofos	1.57 (0.15)^{f}	95.33 (3)	12.76 (1.25)^{e}	60.38 (2)	27.17 (2.71)^{c}	24.34 (1)
Lambda-cyhalothrin	2.80 (0.76)^{e}	91.67 (3)	8.28 (1.10)^{d}	74.29 (2)	17.72 (1.70)^{e}	50.65 (2)
Hexaflumuron	24.19 (2.73)^{c}	28.05 (1)	18.80 (0.76)^{c}	41.63 (2)	21.40 (0.23)^{d}	40.41 (2)
Chlorantraniliprole	23.66 (1.07)^{c}	29.63 (1)	17.58 (0.25)^{c}	45.42 (2)	21.06 (3.78)^{d}	41.35 (2)
Azadirachtin	28.88 (3.13)^{b}	14.10 (1)	27.00 (1.12)^{b}	16.18 (1)	32.46 (2.87)^{ab}	9.61 (1)
Water (negative control)	33.62 (0.96)^{a}		32.21 (3.07)^{a}		35.91 (1.31)^{a}	

Cotton Yield

There were differences in yield and green boll weight between *T. evanescens* (TE) released in combination with pesticides and control plots at the conclusion of each season (Table 3). The yield of cotton varied from 2.3 to 10.3 Ken./fed. in different treatments during the both years. The treatment with azadirachtin and TE had the highest yield of 10.3 Ken./fed. which was followed by hexaflumuron or chlorantraniliprole with TE (8.4 and 8.2 Ken./fed., respectively). Profenofos with TE 2.6 Ken./fed. cotton yielded significantly low than lambda-cyhalothrin and spinosad. Highest healthy green bolls and cotton yield was observed in the treated plots especially after TE release.

Discussion

Several studies have focused on the pesticides potential to control pink bollworm [4, 5], but this pest has developed resistance to pesticides, which has led to an increased demand for non-chemical control approaches against this pest [6]. Nevertheless, few works are available addressing the compatibility and mixture of these pesticides with biological control agents. This study revealed that all the tested treatments effective to suppress of pink bollworm infestation compared to control plots.

Statistically significant differences were recorded between treated plots before and after *Trichogramma evanescens* (TE) release, as the release of the TE in combination with pesticides resulted in a significant decrease in larvae pink bollworm resulting in an increase in cotton yield. Thus, pairing of pesticides with TE can increase efficacy compared to the use of either alone. In a similar studies, Lundgren *et al.* [14] found that reducing the dose of organic and synthetic pesticides used with increasing their effectiveness in the presence of natural parasite *T. brassicae* to control cruciferous Lepidoptera. Hewa-Kapuge *et al.* [15] found that seven pesticides with *T. brassicae* effective in controlling *Helicoverpa punctigera* (Wallengren) and resulted in a high crop yield. Vianna *et al.* [16] reported that family Trichogrammatidae significantly reduced the populations of lepidopteran pests and the number of insecticide applications on tomato crops. Jamshidnia *et al.* [17] and Viteri *et al.* [18] also found that tested pesticide suitable candidate for combination with other Trichogramma spp., towards an integrated management of the *Tuta absoluta* (Meyrick) and *Heliothis virescens* (Fabricius).

Table 3 Average green cotton bolls weight and cotton yield during seasons 2020 and 2021 after applying six pesticides either with or without the parasite *Trichogramma evanescens*

Treatment	Cotton yield (Ken./fed.) [a]	Cotton boll (boll/g)
Spinosad	2.8	2.441 (0.0050)[fg]
+ TE [b]	3.2	2.475 (0.0015)[f]
Profenofos	2.3	2.306 (0.0004)[i]
+ TE [b]	2.6	2.344 (0.0019)[i]
Azadirachtin	8.4	2.874 (0.0011)[b]
+ TE [b]	10.3	2.966 (0.0017)[a]
Hexaflumuron	6.4	2.618 (0.0042)[d]
+ TE [b]	8.4	2.734 (0.0023)[c]
Chlorantraniliprole	6.2	2.533 (0.0021)[e]
+ TE [b]	8.2	2.615 (0.0040)[d]
Lambda-cyhalothrin	2.4	2.362 (0.0067)[h]
+ TE [b]	2.7	2.400 (0.0013)[g]
TE [b]	4.5	1.953 (0.0022)[j]
Water (negative control)	1.9	1.877 (0.0095)[k]

Mean ± SE followed by the same letters are not significantly different (p = 0.05)

[a] Kentar=157.5 kg, [b] *Trichogramma evanescens.*

Based on these results, the pesticides could be divided into three groups. The first group consists of azadirachtin had harmless effects on TE parasitism. Lyons *et al.* [19] and Almeida *et al.* [20] reported few side effects of azadirachtin on Trichogramma spp., which confirmed the results of the present study.

The second group were slightly harmful to TE and consists of chlorantraniliprole and hexaflumuron. Zhao *et al.* [21], Deshmukh *et al.* [22], Afshari *et al.* [23], and Mahankuda *et al.* [24] reported that the recommended field concentration of chlorantraniliprole and hexaflumuron was slightly harmful to *T. chilonis*, *T. pretiosum*, and *T. japonicum* which is the same as these findings in this study.

Finally, the third group, consisting of spinosad, lambda-cyhalothrin, and profenofos, was moderately harmful to TE. Previous studies indicated adverse effects of organophosphates such as acephate, chlorpyriphos, dichlorvos, diazinon, profenofos fenitrothion, phoxim, profenofos, and triazophos were observed on *T. japonicum* and *T. brassicae* [21, 25-26]. Furthermore, moderately harmful effects of pyrethroids (cyhalthrin, cypermethrin, fenpropathrin, fenvalerat, and lambda-cyhaothrin) as well as spinosin (spinosad) have been observed on different Trichogramma species [21, 25, 27-28].

Therefore, according to the results, (i) azadirachtin, (ii) chlorantraniliprole and hexaflumuron, and (iii) spinosad, lambda-cyhalothrin, and profenofos can control pink bollworm by release TE. On the other hand, these results showed that TE is a potential biocontrol agent and can be incorporated into an IPM program with azadirachtin against pink bollworm as an alternative to chemical pesticides while chlorantraniliprole and hexaflumuron should be used with greater care as a part of an IPM procedure. Spinosad, lambda-cyhalothrin, and profenofos, which are extremely harmful to *T. evanescens* (TE), should be avoided.

Conclusion

Overall, the findings indicated that *Trichogramma evanescens* was slightly to moderately adversely affected by hexaflumuron, chlorantraniliprole, spinosad, lambda-cyhalothrin, and profenofos. By contrast, azadirachtin was harmless to *T. evanescens* parasite, thus being recommended as safe insecticide for integration with parasitoid use in pest control programs. This results important for control the pink bollworm effectively, it is necessary to combine biological control agent *T. evanescens* with azadirachtin within integrated pest management strategy in cotton fields in Egypt.

Author contribution

S.M.I. subject selection, study design, carried out the experiments, paper writing, collecting, interpretation of the data, and performing statistical analysis. The author read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

ORCID

Seham Mansour Ismail: https://www.orcid.org/0000-0002-4885-7383

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