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Non-chemical Control of the Pink and Spiny Boll worms in Cotton Fields at Assuit Governorate, Upper Egypt, II- Utilization of the Egg Parasitoid, *Trichogrammatoidea bactrae* Nagaraja

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ABSTRACT

Field trials were carried out to evaluate the efficacy of releasing the egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) at two different application dates (flowering and boll formation cotton plant growth stages) and at different rates of releases (one to four releases) for suppressing the cotton bollworms; the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) and the spiny boll worm (SBW), *Earias insulana* (Boisd.) infestations in two successive cotton growing seasons; 2013 and 2014 at Elwan district, Assuit Governorate (Upper Egypt). Additionally, the average weight of cotton green boll/gm at each treatment plus control was estimated. Generally, data revealed that different rates of releases at the two different application dates showed a significant reduction in % of infestation in green bolls; with PBW (12.54 to 66.36 and 43.74 to 90.03%) and (18.95 to 62.17 and 59.1 to 93.03%) with SBW as compared to control (untreated) in 2013 and 2014, respectively. Moreover, 4-releases early at (> 50%) flowering stage succeeded to suppress the infestation with PBW by (66.36 and 90.03%) in the two seasons, respectively. For SBW, the 4-releases caused a higher reduction in the infestation in the 2nd season (93.09%), opposed to (50%) in the 1st one. Thus, 4-releases of the parasitoid caused (58.18 and 91.56%) of the overall mean of reduction in populations of the two cotton pests throughout the two cotton seasons, respectively. Obtained results showed that both of the control (1.999 gm) and one release (2.075 gm) of the parasitoid through the fruit formation stage had the smallest weight of boll as compared to the rest of the treatments that ranged between (2.428-2.996 gm). Therefore, 4-releases of parasitoid early through the flowering growth stage achieved the best results in reducing the % of pest infestation and high yield.

Key words: Cotton, *Pectinophora gossypiella*, *Earias insulana*, *Trichogrammatoidea bactrae*, Release, Egypt.

INTRODUCTION

Cotton (*Gossypium* spp.) is one of the most important economical crops in Egypt as in many other countries that employed in several related industrial productions. The cultivated area of cotton in Egypt has decreased annually; because of the high costs of agricultural operations, pest control and yield handpicking, accompanied with a low price of seed cotton yield that does not cover the costs of its production (Aziz, 2011).

Insect pests are the most limiting factors that contribute to decrease the cotton production and cause severe damage to the crop yield (El-Heneidy *et al*., 2015). The damage of fruits (green bolls) is frequently more destructive than the other parts of the plant. Cotton bollworms; the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) and the spiny bollworm (SBW), *Earias insulana* (Boisd.), are the most destructive pests of cotton in Egypt (Amin and Gergis, 2006). In Egypt, almost 70% of the total amount of insecticides used for controlling pests in all crops combined, is used only in cotton fields (El-Heneidy *et al.,* 2015). Mostly, farmer relies upon chemicals to get rid of these serious pests, but pesticides have not provided a long term solution for their control. A sharp decline (70– 80%) in the numbers of predatory species occurred in cotton field post chemical applications was reported by El-Heneidy *et al. (*1987**)**. As opposed to this approach, biological control is a major component used worldwide in pest management. It has been considered as a sustainable, economic, environmental friendly and host specific; also, the probability of the host for developing pesticide resistance is limited (Bale *et al.,* 2008).

Mass-rearing and release of natural enemies represents an important tactic of IPM strategy and it has successfully been used to combat many pests including cotton bollworms (Cock, 1985). Among certain natural enemies, the egg-parasitoids of the genus *Trichogramma* are the most widely group used as biological control agents because they easy to rear in insectaries and have vigorous parasitism on different eggs of target hosts, especially their colossal abilities to parasitize the eggs of bollworms in cotton (Ahmad *et al.,* 1998).

The egg parasitoids, *Trichogrammatoidea* spp. have been used in IPM of cotton for the control of *P. gossypiella* and proved as good biological agents in the laboratory (Malik, 2000). Several studies revealed the role of *Trichogramma* spp. in controlling different insect pests infesting the cotton crop in different parts of the world (Charles *et al.,* 2000 and Nadeem *et al.,* 2009). In Egypt, Mesbah *et al*. (2003) evaluated the

efficiency of releasing *T. bactrae* to control the cotton boll worms; PBW and SBW, compared to insecticides, in cotton fields. Parasitoid releases proved best results in reducing PBW infestations in the fallen cotton flower buds and/or squares as well in the green bolls compared to both insecticides and check treatments. Moreover, El-Agamy *et al.* (2011) found that releasing of *T. evanescens* in two waves gave reductions in PBW and SBW larvae ranged between (31.7-44.8 and 23.3-36.7%), respectively in the three successive cotton seasons of 2008, 2009 and 2010.

This study was a trial to find out the most proper timing and number of releases of the egg parasitoid, *Trichogrammatoidea bactrae* Nagaraja, as a bio-control agent, for suppressing the bollworms (PBW and SBW) population in the cotton fields at Assuit Governorate. This is the first field trial of releasing this parasitoid species in the cotton fields at Assuit Governorate, Upper Egypt.

MATERIALS AND METHODS

Experimental trials were carried out to evaluate the efficacy of releasing the egg parasitoid, *T. bactrae* at two different application stages (> 50% flowering and boll formation cotton growth stages) and different numbers of releases (one to four releases) to control the cotton boll worms; PBW and SBW in the two successive cotton growing seasons 2013 and 2014 at Elwan district, Assuit Governorate (Upper Egypt). Three experimental plots, cultivated with the cotton variety Giza 90, were sown on 25th and 16th of March, 2013 and 2014, respectively, following Egyptian clover. The two main plots (A) and (B), one feddan (4200 m^2) each, were treated with *T. bactrae*. The parasitoid was obtained from the Center of Bio-organic Agricultural Services (CBAS) in Aswan and mass-reared on eggs of the Angoumois grain moth, *Sitotroga cerealella* in the laboratory. Its colony was maintained under the laboratory conditions of 25±1°C and 60±10% R.H. To prepare the parasitoid for releasing, eggs of *S. cerealella*, were glued on white paper cards (15x10 cm.) and exposed to parasitoid adults for 24 h to avoid super-parasitism and then the cards were removed and maintained in a refrigerator until needed. The parasitoid was released in the experimental plots inside thick paper envelopes (5x8 cm) (at the rate of about 48 000 parasitoid individuals/ feddan) to protect them from predators' attack and unfavorable weather conditions. To minimize the labor cost, each envelope, contained three strips (1x1 cm.) of different stages of parasitized eggs, was hanged manually on the cotton leaves at about (50 cm) above the ground (Abd El-Rahman *et al.,* 2008). Releases were carried out early in the morning or late in the afternoon to avoid the heat of the noon time. The two main plots were further divided into four subplots (¼ feddan each = 1000 m^2). Each subplot was separated from the adjacent one by at least 100m (as a barrier) to avoid interference between treatments. Different release treatments were evaluated at each plot by:

- **Timing** (starting late in June (plot A) and mid-July (plot B)).
- **Rate of releases** (24 cards about 1500-2000 parasitoids/card/feddan, with a total of 40000 – 48000 parasitoids/ feddan). Cards were hanged on the plants as release points every 14m and were about 7 m to the inside of the edges of the field.
- **No. of releases** (once, 2, 3 and 4 times).
- **Intervals** (10 days).

The third plot (C), the control was located about 1Km away from the treated fields to reduce the possibility of parasitoid dispersal and was left without releases and/or insecticidal treatments during the study. Release and control plots were also separated by a corn field, providing an additional barrier against the movement of releasing parasitoid into control plot. Experimental plots received regular agricultural practices.

Weekly random samples of green cotton bolls (100 bolls/ sample) were collected from each plot (treated and control) during July-September (11-weeks). The boll infestation rate was estimated compared to the control plot. Percentages of infestation with PBW and /or SBW were estimated by dissecting the green bolls at the same day of collection. Infestation records were based on the existence of injury symptoms regardless to the presence of the larvae.

$$\text{Infestation \%} = \frac{\text{No. of infested bolls}}{\text{Total no. of collecting bolls}} \times 100$$

By the end of each season, the yield was estimated in both treated and untreated fields, also four randomly selected areas were hand harvested for checking the boll weight in all experimental trials.

Obtained data were recorded and statistically analyzed using one and three-way ANOVA by the Advanced Statistical Analysis Package (ASAP)^R (Darwish *et al.*, 2012).

RESULTS AND DISCUSSION

Season 2013

Just a pre - release of the parasitoid, percentage of infested bolls with PBW was (4 and 3%) in the control (untreated) and the experimental areas, respectively. On the contrary, for SBW, no infestation was observed in both areas. The first incidence of PBW in green bolls was recorded in July at all treatments in plot (A), while it was (2%) at the 2nd

fortnight of July in the untreated area only in plot (B). In contrast, the first incidence of SBW was noticed by late August, in untreated and treated areas. On both dates of application, the rate of infested bolls increased towards the end of the season (harvest time) reaching its maximum in September (20-36.66 & 14.67-18% in plot (A) and 32-42.5 & 10-17.5% in (B)) for PBW and SBW, respectively, however, it was low as compared to the untreated (44 and 21-25.34%) for both pests in all treatments; indicating that the parasitoid succeeded to suppress the infestation rate. Generally, bollworm infestation at late season (August-September) was much higher than that in the early season (June-July). Based on obtaining results (Fig. 1), the 4-releases of *T. bactrae* were able to reduce the total percentage of boll worm infestation than control with about (58.18 and 53.98%) during the two application dates, respectively. Additionally, releases of *T. bactrae* at the boll-formation growth stage showed an increasing role against SBW than PBW.

Season 2014

It was noticed that the percentage of infestation with both of PBW and SBW during this cotton season was much lower than that recorded in 2013. Pre-releasing the parasitoid, the % of infested bolls with PBW was (4 and 2.67%) in the control and the experimental areas, respectively. On the contrary, no infestation was observed in both areas with SBW. Data showed that the highest infestation rate by both pests in the green bolls during this season was recorded in all treatments in August, with different rates. It was obvious that 4-releases of *T. bactrae*, when applied at the (>50%) flowering growth stage succeeded to reduce the total percentage of bollworm infestation than control with (91.56%), opposed to (80.26%) during the fruit formation growth stage throughout the whole period of the season (Fig. 2). Also, these results proved that releases of this parasitoid early or late at the (50%) flowering or fruit formation growth stages showed an efficient role against SBW than PBW, when compared to control. This may be attributed to the presence of okra plants (major host plant) in the adjacent areas and the increase infestation rate of SBW in this season.

As indicated in table (1) and figs. (1 & 2) statistical analysis ($P<0.01$) of the results in both cotton growing seasons 2013 and 2014 concluded that:

1- Early-release of *T. bactrae* at (> 50%) cotton flowering growth stage (late-June) showed highly significant differences in the percentage of infestation and total reduction compared to that of the late release during fruit formation stage (mid-July).

   Regardless of the two different dates of applications, % of infestation with PBW and SBW revealed highly significant differences.

3- Number of releases showed highly significant differences in % of infestation with PBW and SBW in both dates of application as compared to control.

4- A three-way analysis of variance of the data (ANOVA) proved that the 4-releases of *T. bactrae* was the most effective treatment for suppressing % of infestation in green bolls with PBW and SBW in relation to the two different dates of applications.

The effective impact of releasing the parasitoid early is in agreement with those of (Naranjo *et al.*, 1992) in Arizona and (Scholz and Murry, 1995) in Australia, who recorded that the potential of *T. bactrae* wasps against PBW was the best when released in the early season. Moreover, in Egypt, previous studies showed that the local or imported species of *Trichogramma* were able to maintain PBW and SBW densities below the economic level of infestation (3%) in most dissected boll samples as recorded by (Abd El-Hafez & Nada, 2000; Abd El-Hafez *et al.*, 2002; Mesbah *et al.*, 2003 and Khidr *et al.*, 2003). El-Heneidy *et al.* (2004) tried to choose the proper timing and number of releases of the egg parasitoid, *T. evanescens*, for controlling PBW and SBW in Egyptian cotton fields at Menoufia and Sharkia Governorates, season 2003. Two to four parasitoid releases were conducted during the flowering and boll formation growth stages. They found that the parasitoid releases showed significant reductions in the percentages of boll worms' infestation comparing to the insecticidal application areas (control). The reduction ranged between (48.6 to 56.5% and 16.4 to 21.7%) when the parasitoid was released early during the flowering stage and a few weeks later during the boll formation growth stage, respectively. Also, in the parasitoid release areas, number of insecticidal applications was reduced to

Table (1): Analysis of variance (3-way ANOVA) for main effects and interactions of application time (A), cotton bollworm species (B) and number of parasitoid releases (C) in suppressing the infestation of green bolls (P = 0.01)

Season 2013							
Source of variation	Main effects			Interactions			
	A	B	C	A x B	A x C	B x C	A x B x C
df	1	1	4	1	4	4	4
F	849.68	2888.98	247.34	839.89	32.69	32.71	35.67
P	**	**	**	**	ns	**	**
$LSD_{0.01}$	1.073	1.073	1.696	1.517	2.399	2.399	3.392
Season 2014							
df	1	1	4	1	4	4	4
F	1878.18	5175.51	246.31	1969.42	18.26	59.28	33.61
P	**	**	**	**	ns	**	**
$LSD_{0.01}$	1.043	1.043	1.649	1.475	2.331	2.331	3.297

A=Date of application (the flowering >50% and boll formation stages), **B=**Cotton bollworm species (PBW & SBW), **C=** Number of releases of parasitoid (1-4 releases), **Significant at 0.01, ns= no significant

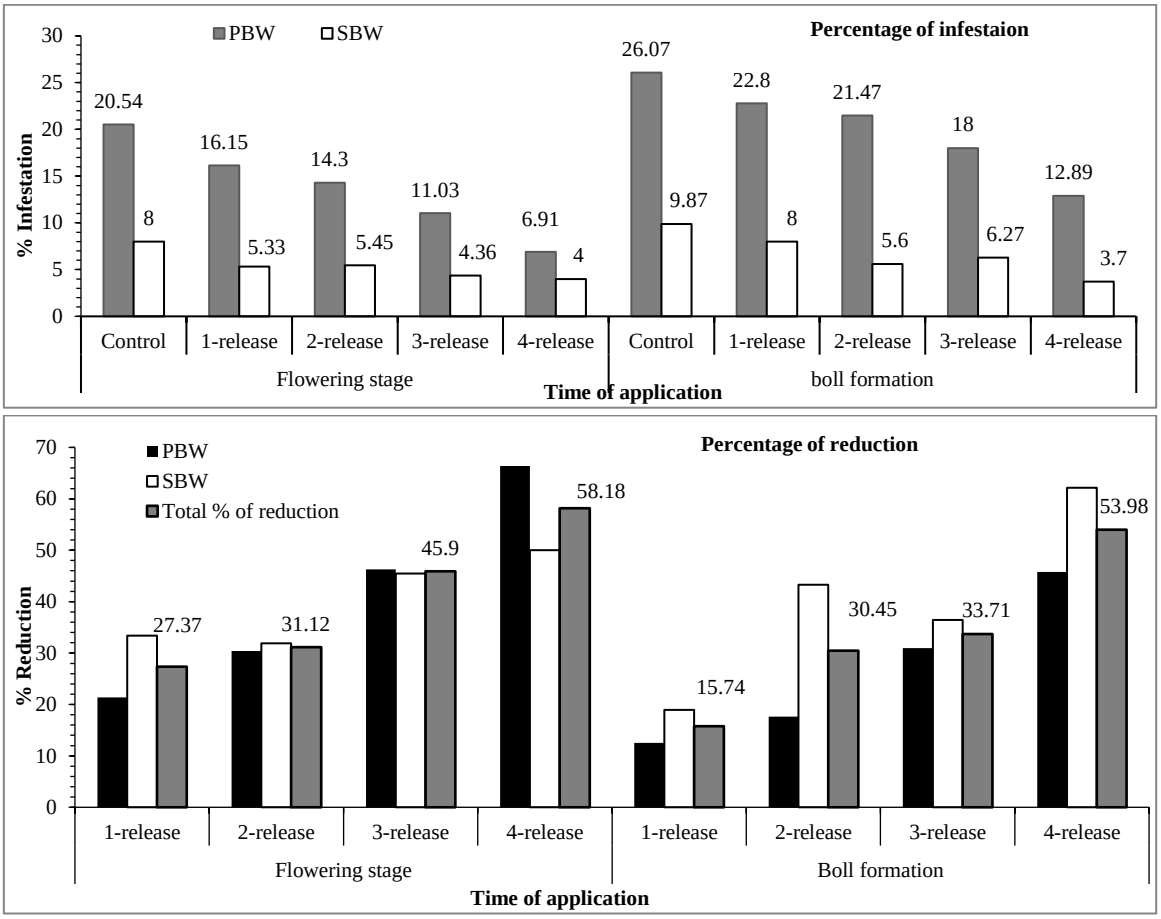

Fig. (1): Efficacy of different releases of *T. bactrae* (one-four releases) in relation to different application dates to suppress PBW and SBW infestation % to cotton green bolls at Assuit Governorate during season 2013.

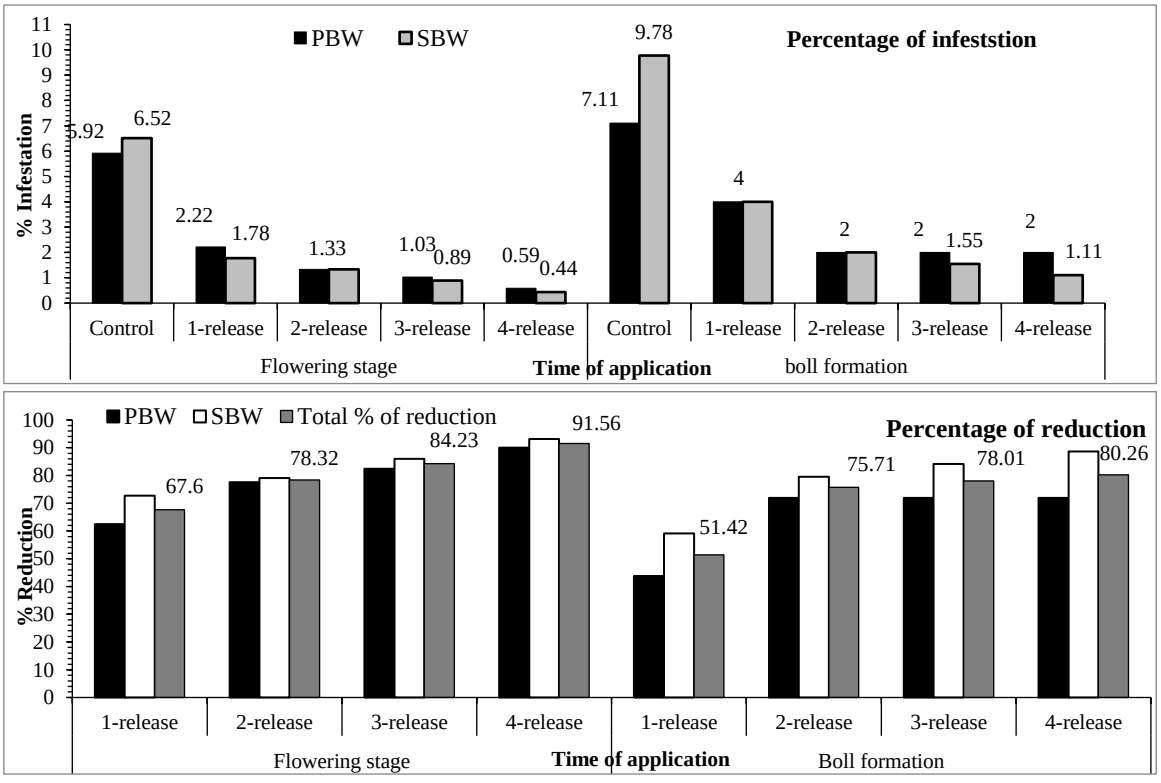

Fig. (2): Efficacy of different releases of *T. bactrae* (one-four releases) in relation to different dates to reduce PBW and SBW infestation % in cotton green bolls at Assuit Governorate during season 2014.

Table (2): Cotton yield in two different dates of releasing *T. bactrae* and in control field at Assuit Governorate in the cotton growing seasons 2013 and 2014

Cotton production/feddan (Kentar=157.5 kg)			
Season	*T. bactrae* (A) (Flowering stage)	*T. bactrae* (B) (Boll formation stage)	Control
2013	9.50	8.25	5.75
2014	10	9	7
Mean	9.75	8.62	6.37

Table (3): Average weight of cotton boll/ gm ±SE, arranged descending, in relation to different treatments in the cotton fields at Assuit Governorate, seasons 2013 and 2014

Treatments	Mean±SE (gm./ boll)
4-releases (A4)	2.996 ± 0.126^{a}
3-releases (A3)	2.840 ± 0.125^{abc}
2-releases (A2)	2.655 ± 0.136^{bcdef}
4-releases (B4)	2.563 ± 0.136^{cdef}
2-releases (B2)	2.484 ± 0.109^{def}
1-releases (A1)	2.481 ± 0.155^{ef}
3-releases (B3)	2.428 ± 0.083^{f}
1-releases (B1)	2.075 ± 0.087^{gh}
Control	1.999 ± 0.094^{h}
LSD 0.05%	0.321

Means in the column followed by the same letters are not significantly different at the 0.05 level of probability.

almost one third and consequently, the costs were dropped by (29.3-36%). Further, Tohamy and Kassem (2007) in the Minia Governorate (Egypt), recorded the highest number of *T. evanescens* applications (5 times) gave the lowest mean number of bollworm larvae (2.5 and 2.8/ 100 green boll) associated with the highest number of open bolls/ plant (11.1 and 9.8), and seed cotton yield (8.0 and 6.8 Kent./fed.) in two successive seasons, respectively. Also, in New Valley Governorate (Egypt), Abd El-Rahman *et al.* (2008) released *T. evanescens* in 2003 and 2004 cotton seasons and found that, 5 releases (17.600 parasitoid females in 2 waves/feddan/release) in 2 week intervals successfully suppressed SBW infestation to be one fourth to one third of that of the untreated fields and successfully decreased the losses in cotton yield. In addition, Baraka *et al.* (2008) applied 5 and 6 releases of *T. evanescens* for suppressing SBW infestation in 2004 cotton season and observed that, 5-releases presented (76.92%) of mean reduction in boll infestation compared to (83.97%) of 6 releases. Also, cotton yield loss was (13.17%) in the five releases field compared to (12.05%) in the six releases one. Recently, Ghanim *et al.* (2010) in Dakahlia Governorate, revealed that, the average reduction of infestation by the two insect pests were (53.91 and 56.88%) in 2008 and 2009 seasons, respectively due to *T. evanescens* release. In Sohag Governorate, Salman *et al.* (2014) studied the efficacy of releasing *T. evanescens* alone or with other predators on PBW and SBW and revealed that all biological control treatments decreased significantly the population of both bollworms. Similarly, in Behaira Governorate, Saad *et al.* (2015) observed that all the treatments either the release of *T. evanescens* alone or in combination with the tested pesticides gave best bollworms control.

Cotton production and boll weight

At the end of each season, the cotton yield per feddan at the two different dates of application (flowering stage **(A)** and fruit formation growth stage (**B**)), versus control was determined (Table 2). It was notable that when the percentage of infestation decreased the cotton yield increased. Additionally, the average weight of cotton bolls at each treatment was estimated (Table 3). Statistical analysis revealed that the control (1.999 gm) and applying one release (2.075 gm) of the parasitoid through the fruit formation growth stage had the lowest weight of boll, with a highly significant difference, as compared to the rest of treatments. This may due to the number and timing of the releases. As well, obtained results throughout the two cotton growing seasons 2013 and 2014 concluded that the treatment of cotton fields with the parasitoid, *T. bactrae*, was sufficient to suppress the infestation with the two cotton bollworms compared to control. Moreover, the higher reduction in the infestation, higher cotton weight, besides greater cotton yields was achieved in case of the four releases early during the flowering growth stage.

The present results recorded higher yield and greater weight of green boll in the parasitoid released area than the control. This field observation is in a similar line with those of (Naranjo, 1993; El-Heneidy *et al.*, 2004; Tohamy & Kassem, 2007; Salman *et al.*, 2014 and Saad *et al.*, 2015).

In conclusion, the egg parasitoid, *T. bactrae* can successfully be used as a biological control agent against both cotton bollworms (PBW and SBW) in cotton fields in Upper Egypt. Furthermore, releasing of this parasitoid 4 times early during the cotton flowering growth stage is recommended based on the obtained promising results. The use of this parasitoid was less expensive (<50%) as compared to the expenses of insecticide spray when both control methods were compared.

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