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BIOLOGICAL CONTROL OF *Pectinophora gossypiella* (SAUND.) AND *Earias insulana* (BOISD.) IN COTTON FIELDS AT DAKAHLIA GOVERNORATE, EGYPT BY AUGMENTATIVE RELEASES OF *Trichogramma evanescens* WESTWOOD

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ABSTRACT

Field studies were conducted at Dakahlia Governorate, Egypt in 2004 and 2005 cotton seasons to evaluate the releases of *Trichogramma* wasps for pink bollworm, *Pectinophora gossypiella* (Saund.) and spiny bollworms *Earias insulana* (Boisd.) management in cotton. In the two seasons, six releases of *Trichogramma evanescens* Westwood 10-15 days intervals were made into 999 and 600 feddan, respectively. While, the recommended bollworms insecticide control program was applied at the control units. This recommended program suggested to use insecticide applications (insect growth regulators, organophosphoric and pyrethroid compounds) when infestation met or exceed the economic level (3%). The overall reduction of infestation with the two pests in *Trichogramma* units averaged 23.73 and 40%, since infestation averaged 1.35 & 1.44.0 in these units and 1.77 & 2.4% in control units at the two seasons, respectively. Meanwhile, *T. evanescens* succeeded to maintain boll infestation below the economic level in 78 and 84% of the dissected boll samples in the two seasons, respectively. The corresponding figures by insecticides were 67 and 56% in the two seasons, respectively.

Keywords: Pectinophora gossypiella; Earias insulana; Trichogramma evanescens; biological control

INTRODUCTION

In Egypt, management of insect pests of cotton depends on the application of chemical insecticides. Use of chemical insecticides induce resistance in insects and destroy the natural pest control agents. Therefore, alternatives to insecticides are receiving much attention. Recently, the Egyptian Ministry of Agriculture and Land Reclamation applying a new strategy for controlling lepidopterous pests depending on releasing *Trichogramma* in cotton and several crops. Egg parasitoids of the genus *Trichogramma* have been widely used as biological control agents throughout the world on numerous agricultural commodities (Smith *et al*., 1986; Tuhan *et al.* 1987; Hassan, 1993; Smith, 1994; Zandigiacome & Greatti, 1997; Abd El-Hafez & Nada, 2000; Abd El-Hafez *et al*., 2002; Shalaby *et al*., 2002; Abd El-Hafez, 2004 and Abd El-Hafez *et al*., 2004 & 2006). Meanwhile, egg-parasitoids are the most efficient in controlling many insects, as these beneficial insects prevent hatching of the host eggs by feeding on the internal contents of eggs, and consequently killing the target pest before eclosion of larvae and causing any kind of damage. In this respect, the true egg-parasitoids when released in sufficient numbers, act in controlling the pest in a similar way of that occurring by ovicides as both cause mortality of the pest

when still in the egg stage, but the egg-parasitoids have the advantage of control without any environmental pollution. The present study was conducted to evaluate a biological program depending mainly on releasing the local egg parasitoid *Trichogramma evanescens* Westwood for controlling pink bollworm, *P. gossypiella* and spiny bollworm *E. insulana* in cotton fields

MATERIALS AND METHODS

A biological program was conducted for controlling pink bollworm, *Pectinophora gossypiella* (Saund.) and spiny bollworms *Earias insulana* (Boisd.) in cotton fields during two successive cotton seasons (2004 and 2005) at Dakahlia Governorate, Egypt. This program depended mainly on releasing the local egg parasitoid *Trichogramma evanescens* Westwood. Six releases were done with a rate of 22 release cards/ feddan (1500 parasitoids/ each). The number of release parasitoids per feddan was increased by increasing bollworms populations. Regardless the time of release, if the infested bolls reached 3%, additional treatment with *T. evanescens* was done immediately and the number of release cards increased to 30 cards/ feddan (45000 parasitoids). If infestation reached 5%, an application with safe insecticides was done (*i.e.* IGR and B.t. formulations). Seven days must be left between insecticide treatment and *Trichogramma* release.

Rearing Technique

T. evanescens (a native parasitoid) was reared on Angoumois grain moth, *Sitotroga cerealella* (Oliv.) eggs according to the method described by Hassan (1993 and 1995). For efficient mass rearing of the parasitoid, *S. cerealella* eggs (< 24h old) were glued to paper cards (21X15 cm.) and exposed to *Trichogramma* adults in glass jars (2-liters capacity) provided with 10% sucrose solution for nutrition and covered with two layers of cloth-wrapped cotton kept in position by rubber band. Egg sheets were renewed daily to avoid super-parasitism and the parasitized egg sheets were kept in clean glass jars. Rearing took place at constant temperature of 25 ± 1°C and 80 ± 5 %R.H (Abd El-Hafez, 1994).

Trichogramma evanescens Release

Releases of *T. evanescens* were made using thick paper cards (Duplex paper, 250g weight) containing parasitized *S. cerealella* eggs. Each card contained three cohorts with parasitoids (about 500/ each) expected to begin emergence within 1, 3 & 5 days after field release. Thus, the total number of parasitoids/ card was about 1500 parasitoids. Six waves of *Trichogramma* adults emerge from each card with a rate of two waves per each stage. The emergence of parasitoids waves begins within 24h after release, and continues through 6- 7 days. On this way, *Trichogramma* adults cover a control period ranged between 9-11 days according to the longevity of the emerged adults (Abd El-Hafez, 1995). In addition, by releasing three developmentally stages of *T. evanescens* at the same time, handling time in the laboratory as well as time spent for releasing cards in the field was greatly reduced, without sacrificing *T. evanescens* emergence from release cards. Twenty-two cotton plants/ feddan were selected to serve as release points, where distances between these points were 14 m and started 7m from the

edges of the field. Cards were hand-placed before the sunset or at the early morning on a 0.5m above soil surface. The releasing cards were transported to the field in a cooling box to avoid the adverse effect of hot weather during transportation.

Trichogramma released areas were 999 and 600 feddan distributed into 43 and 22 units, at 2004 and 2005 cotton seasons, respectively. These units were selected from El-Cemblaween, Aga, El-Mansoura, Talkha, Sherbeen, Meat Sewead, Meniat Al-Nasr, Dekernes, El-Manzala, Belkas, Temai Al-Amdeed and Meat Ghamr regions (12 regions) at the first season and from El-Cemblaween, Aga, El-mansoura, Sherbeen, Meat Sewead and Belkas regions (6 regions) at the second one. All fields were prepared for cotton planting using conventional practices for that area, and planted with cotton variety Giza 86. The planting date for both years at the selected units was between 20-30 April. Six *Trichogramma* releases were applied in these units at about 10- 15 days intervals. Releases were made on 28 June, 8, 18, 28 July and 10, 25August at the first season and on 22 June, 3, 12, 27 July and 10, 25August at the second one.

Control Treatments:

Control plots were sprayed by insecticides when infestation with bollworms was met or exceeded the recommended threshold of bollworms insecticide treatments in Egypt (3% of infested bolls or a catch of 8 moths/ 3 nights/ trap). When inspection suggested treatment, recommended compounds such as IGR (Atabron & Cascade), organophosphorous (Curacrone, Bestiban & Teleton) and pyrethroid compounds (Somi-gold, Kendo & Karat) were applied in recommended dose and sequence design using a knapsack motor spryer. This resulted in 4 applications spaced 10-15 days in both years. Additionally, all applications were made when there was no measurable wind, so that the possibility of insecticide drift into release and control plots was negligible

At the two seasons, *Trichogramma* release units were separated by a minimum of 300m from the control units (insecticide treated) to reduce possibility of parasitoid dispersal into these units. The sequential sampling method was used to evaluate the infestation of green cotton bolls with pink and spiny bollworms. Therefore, three samples (100 green bolls/ each) were collected randomly from *Trichogramma* and control units at 6 days intervals, dissected and the number of infested bolls was recorded. On the other hand, samples were divided into four categories according to the infestation level *i.e.,* the first category include samples free from any infestation, the second category include samples which have 1 infested bolls, the third category include samples which have 2 infested bolls and the fourth category include samples which have 3% and more infested bolls. Reduction in infested bolls in *Trichogramma* release units was calculated according to the following equation:

$$\% \text{ Reduction} = \frac{C - T}{C} \times 100$$

Where C: the estimated parameter in control (insecticide treatments)

T: the same parameter in *Trichogramma* release units

While analyses of variance (ANOVA) were conducted on data of the two seasons and when statistical difference existed within a data set, Duncan's multiple range tests was used to separate the means (Snedecor & Cochran, 1980).

Quality Control

To ensure that high quality parasitoids were used throughout the experiment, field and laboratory quality control samples were taken from each released cards. For each release, five cards were maintained in the laboratory to estimate production emergence and sex ratio. In addition, twenty cards were selected at random (ten days after each release) and recovered to the laboratory where the percentage of emergence was determined by sampling 50 black eggs per card for adult emergence holes. Data reveal optimal levels of emergence under field conditions which are similar to those recorded in laboratory (91-98%). Also, high percentage of females in progeny (61.67- 72.0% females) was calculated.

RESULTS AND DISCUSSION

2004 cotton season. The first release of *Trichogramma* was done at June 28th, while the first green cotton boll samples were available at July 9th in 35 units from 43 ones. Few numbers of green cotton bolls were infested with spiny bollworm (SBW) throughout the whole season comparing with pink bollworm (PBW). The percentages of infestation with SBW ranged between 0- 0.14% with an average of 0.03% in *Trichogramma* release units, while they ranged between 0.03- 0.17% and averaged 0.09% in insecticide treatments. Table (1) shows the mean percentages of the total infestation with the two pests in the sequential samples. In the first inspection, 10 infested bolls with PBW were recorded from *Trichogramma* samples (35 samples) to give 0.29% infestation opposed to 0.75% in the insecticide treatments indicating 61.33% reduction in infested bolls due to *Trichogramma* release. In the second inspection (July 15th), 14 infested bolls with PBW were recorded from *Trichogramma* treated units (43 samples) to give 0.33% infestation opposed to 0.95% in control area. This percentage was less than in insecticide treatments by 66.33%. The 3rd release of *Trichogramma* was done at July 18th. In the following inspection (July 21st), an increase in the boll infestation occurred in both the control (insecticide) and *Trichogramma* samples as they averaged 1.24 and 0.64%, respectively, indicating 48.39% reduction in the percentage of infested bolls due to *Trichogramma* release. The 4th release of *T. evanescens* occurred at July, 28th. At this time, the total percentage of infestation with pink and spiny bollworms averaged 1.05 and 1.64 % in *Trichogramma* and insecticide treatments, respectively, indicating 35.98% reduction in area that received *T. evanescens* release than those treated with insecticides (control). Percentage of infested bolls in *Trichogramma* units increased to 1.11 at the 5th inspection, indicating 42.19% reduction than insecticide treatments (1.92%). On the contrary, infestation in *Trichogramma* units (2.26%) became over that in insecticide units (2.18%) by 3.67% in the six inspection. The 5th *Trichogramma* release was applied at August 10th. On

August 14th (7th inspection), the percent of infestation decreased to 1.74% in *Trichogramma* units opposed to 2.41% in control units, thus 27.8% reduction was achieved as a result of *Trichogramma* release. Slight increase in infestation occurred in *Trichogramma* and insecticide units at the following inspection as they reached 1.93 and 2.47%, respectively. The reduction induced by *Trichogramma* was determined by 21.86%. The 6th *Trichogramma* release occurred at August 25th. At the 9th inspection, percentage of infested bolls in Trichogramma units (2.07) became below that in control units (2.16%) by 4.17%. At the 10th inspection, infestation increased in *Trichogramma* units (2.09%) to become over that in insecticide units (1.90%) by 10%. The last inspection was at September 7th. At this time, the percent of infested bolls averaged 1.39 and 1.81% in *Trichogramma* and insecticide treatments, respectively, while the reduction was 23.20% in *Trichogramma* units. At the end of the season, the estimated whole percentage of infestation averaged 1.35% in *Trichogramma* release units opposed to 1.77% in insecticide units. Accordingly, 23.73% reduction was achieved due to *T. evanescens* release.

Table 1. Infestation of green cotton bolls with pink (PBW) and spiny bollworms (SBW) in *T. evanescens* release and insecticide-treated units at Dakahlia Governorate through 2004 cotton season

Inspection	Date	Infestation (%)						% Reduction (+/ -)
		Insecticides			*T. evanescens*			
		PBW	SBW	Total	PBW	SBW	Total	
1st	July 9th	0.70	0.05	0.75	0.29	0.00	0.29	61.33
2nd	July 15th	0.92	0.03	0.95	0.33.	0.00	0.33	66.31
3rd	July 21st	1.20	0.04	1.24	0.57	0.07	0.64	48.39
4th	July 27th	1.55	0.09	1.64	0.91	0.14	1.05	35.98
5th	Aug. 2nd	1.75	0.17	1.92	1.11	0.00	1.11	42.19
6th	Aug. 8th	2.07	0.11	2.18	2.19	0.07	2.26	-3.67
7th	Aug. 14th	2.37	0.04	2.41	1.74	0.00	1.74	27.80
8th	Aug. 20th	2.38	0.09	2.47	1.86	0.07	1.93	21.86
9th	Aug. 26th	2.03	0.13	2.16	2.07	0.00	2.07	4.17
10th	Sept. 1st	1.79	0.11	1.90	2.02	0.07	2.09	-10.00
11th	Sept. 7th	1.70	0.11	1.81	1.39	0.00	1.39	23.20
Mean		**1.68**	**0.09**	**1.77**	**1.32**	**0.03**	**1.35**	**23.73**

ANOVA yielded no significant difference between the total percentages of infestation with bollworms in *T. evanescens* and insecticides treatments (F= 2.651).

Regarding the green boll infestation throughout the 11 inspections (Table 1 & Fig.1), it could be noted lower infestation in *Trichogramma* samples at all inspections than in insecticide ones (control) except at the 6th and 10th inspections. The increase of infestation at the two aforementioned inspections may be due to releasing insufficient numbers of parasitoids that were unable to suppress the natural increase of the target populations, which always occurred at this time. Thus, it is recommended to increase the number of released parasitoids and to decrease the period between releases.

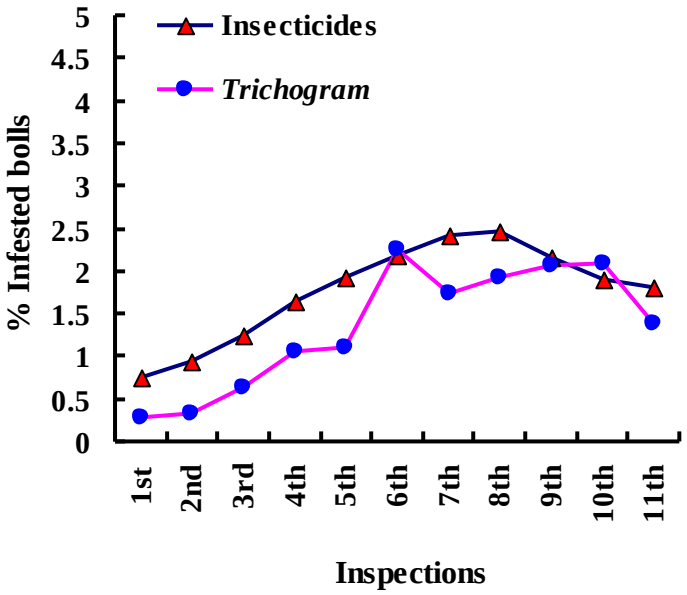

Fig.(1): Infestation with pink and spiny bollworms in *Trichogramma* release units comparing with those treated with insecticides at Dakahlia Governorate (2004 cotton

The illustrated data in Fig. (2) revealed that 44% of samples from *Trichogramma* units (464 samples) were free from any infestation while 18 and 16% of these samples have 1 and 2 infested bolls, respectively. Meanwhile 22% of these samples have 3 and more infested bolls. As for samples from insecticide treatments (control), 29% of these samples were free from any infestation while 17 & 21% have 1 and 2 infested bolls, respectively. On the other hand, 33% of the samples have 3 and more than three infested bolls. Thus, *T. evanescens* succeeded to maintain pink and spiny bollworm densities below the economic level of infestation (3%) in 78% of these samples, since 22% of the samples have 3 and more infested bolls. As for control, the four applications with chemical insecticides were able to maintain these densities below the economic level of infestation in 67% of the dissected samples, while 33 % of the whole samples have 3 and more infested bolls. Statistical analyses of data showed no significant difference between the infestation in *Trichogramma* and insecticide treated units (F value = 2.651).

2005 cotton season. In this season, *T. evanescens* was released for 6 times (June 22nd - August 25th) in 600 feddan distributed at 22 units. Table (2) shows the percentages of boll infestation with pink and spiny bollworms in all inspections throughout the whole season (10 inspections; 22 samples/ each and 100 bolls/ sample). Two releases with *T. evanescens* were done before availability of the first boll sample. Meanwhile, the 3rd release of *Trichogramma* was at the same time of the first inspection (July 12th).

Regarding the infestation with SBW in *Trichogramma* release units, it could be noted that 4 inspections were free from SBW infestation. The SBW infestation ranged between 0- 0.23% with an average of 0.08% in *Trichogramma* release units and between 0.05- 0.80% with an averaged of 0.29% in insecticide treatments.

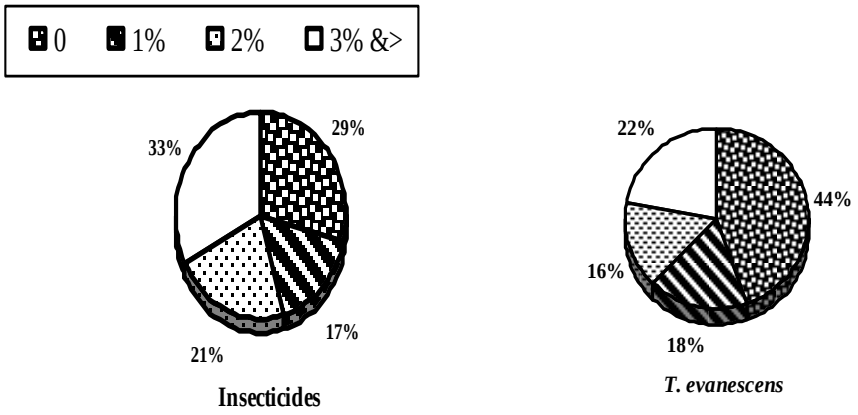

Fig.2. Categories of the infestation levels of all samples, collected from *Trichogramma* released and insecticide-treated units throughout 2004 cotton season in Dakahlia Governorate.

Regarding the results in Table (2) and Fig.(3), it could be noted that the infestations with the two pests in all inspections of *Trichogramma* release units were below those of insecticide ones; the reduction percentage ranged between 9.09- 83.93. At the first three inspections, percentages of infestation were 0.27, 0.23 and 0.36% in *Trichogramma* release units opposed to 1.68, 0.99 and 1.21% in insecticide units, respectively. The 4th release of *Trichogramma* was done at July 27th. In the following inspection total infestation with the two pests averaged 0.9 and 1.39% in *Trichogramma* and insecticide units, respectively, showing 35.25% reduction due to *T. evanescens* release. On August, 5th, these percentages increased to 1.32 and 1.87%, respectively, so reduction percentage declined than the previous inspection to reach 29.41%. The 5th *Trichogramma* release applied at August 10th, while the 6th inspection was at August 11th. In this inspection, 50.65% reduction in infestation was achieved as a result to the effect of *Trichogramma* opposed to increase of infestation in control to reach 2.31%. The subsequent sample of August 17th, showed that infestation with pink bollworm increased to 3.10 and 3.41% in both *Trichogramma* and control units, respectively; *i.e*. *T. evanescens* achieved 9.09% reduction in

infestation. In the 8th inspection, *Trichogramma* release (1.86%) caused decrease in the percentage of infestation to be below that of control (3.47%) by 46.4%. The 6th *Trichogramma* release occurred at August 25th. On August 29th, infestation averaged 1.95 and 3.80% in *Trichogramma* and control units, respectively, showing 48.68% reduction due to *Trichogramma* release. In the last inspection, the infestation in *Trichogramma* units (3.25%) was below that of control units (3.85%) by 15.58%. On the other hand, the overall reduction of infestation with the two pests in *Trichogramma* units averaged 40%, where infestation averaged 1.44 and 2.4% in *Trichogramma* and control units, respectively (Table 2 & Figs. 3). Statistically *Trichogramma* was more effective in reducing infestation than insecticides (F value = 4.541 & LSD= 0.9397)

Table 2. Infestation of green cotton bolls with pink (PBW) and spiny bollworms (SBW) in *T. evanescens* release units comparing with insecticide-treated units at Dakahlia Governorate through 2005 cotton season

Inspection	Date	Infestation (%)						% Reduction
		Insecticides			*T. evanescens*			
		PBW	SBW	Total	PBW	SBW	Total	
1st	July 12th	1.55	0.13	1.68	0.27	0.00	0.27	83.93
2nd	July 18th	0.94	0.05	0.99	0.23	0.00	0.23	76.77
3rd	July 24th	1.08	0.13	1.21	0.36	0.00	0.36	70.25
4th	July. 30th	1.20	0.19	1.39	0.85	0.05	0.90	35.25
5th	Aug. 5th	1.66	0.21	1.87	1.23	0.09	1.32	29.41
6th	Aug. 11th	2.12	0.19	2.31	1.05	0.09	1.14	50.65
7th	Aug. 17th	3.15	0.27	3.41	3.10	0.00	3.10	9.09
8th	Aug. 23rd	3.14	0.33	3.47	1.72	0.14	1.86	46.40
9th	Aug. 29th	3.24	0.56	3.80	1.77	0.18	1.95	48.68
10th	Sept. 4th	3.06	0.80	3.85	3.02	0.23	3.25	15.58
Mean		**2.11**	**0.29**	**2.40**	**1.36**	**0.08**	**1.44**	**40.00**

ANOVA yielded significant difference between the total percentages of infestation with bollworms in *T. evanescens* and insecticides treatments (F= 4.541 & LSD= 0.9397).

Results illustrated in Fig. (4) show that 48% of samples from *Trichogramma* treated units were free from any infestation opposed to 21% in those from insecticide treated units. Meanwhile, 19 & 16% and 15 & 20% of the samples from the two treatments have 1 and 2 infested bolls, respectively. So, *T. evanescens* succeeded to maintain pink and spiny bollworm densities below the economic level of infestation in 83% of the samples since 17% of the whole samples had 3 or more infested bolls. As for control, the four applications with insecticides were able to maintain these densities below the economic level of infestation in 56% of the dissected samples, while 44% of samples had 3 and more infested bolls.

The present results indicated that *T. evanescens* is a good tool for controlling pink and spiny bollworms in cotton fields. Similar results are reported by many authors. For example, Tuhan *et al.* (1987) released *Trichogramma brasilience* in India at a rate of 20 000 newly emerged adults/acre per week in combination with sprays of carbaryl, dimethoate and monocrotophos in cotton fields. They found that these treatments

significantly, reduced the damage caused to cotton by *Earias insulana*, *E. vittella* and *P. gossypiella*. In China, Chao *et al.* (1996) released *T. flavum* in 1993-95, in Nanpi County, to control cotton bollworms (Noctuidae) and found that the release of *T. flavum* was less costly than chemical sprays and increase yield of cotton. At the same time, natural enemies were protected, and environmental pollution was avoided. They added that, a small release of *T. flavum* was sufficient to control the pests' population during the year of moderate/light incidence of Noctuidae. However, during an outbreak year, both releases of both *T. flavum* and spraying chemicals were necessary. They recommended using microbial pesticides to minimize harmful effects to *T. flavum*. Moreover, Cheema *et al*., (2004) stated that *T. chilonis* destroyed eggs of lepidopteron moths biologically in different genotypes of cotton crop in Pakistan. They suggested that it can be added as cotton insect pest management tool. In Egypt, previous studies show that the local or imported species of *Trichogramma* were able to maintain pink and spiny bollworm densities below the economic level of infestation in most dissected boll samples (Abd El-Hafez & Nada 2000; Abd El-Hafez *et al*. 2002; Shalaby *et al.* 2002; Abd El-Hafez 2004 and Abd El-Hafez *et al*. 2004 & 2006).

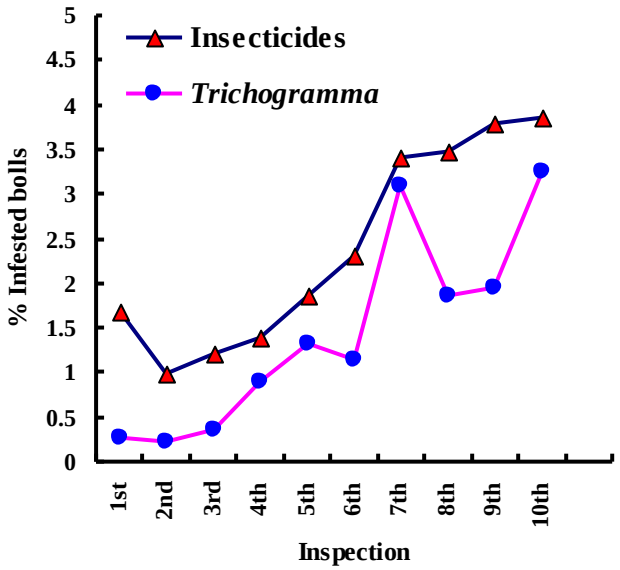

Fig.(3): Infestation with pink and spiny bollworms in *Trichogramma* release units comparing with those treated with insecticides at Dakahlia Governorate (2005 cotton season)

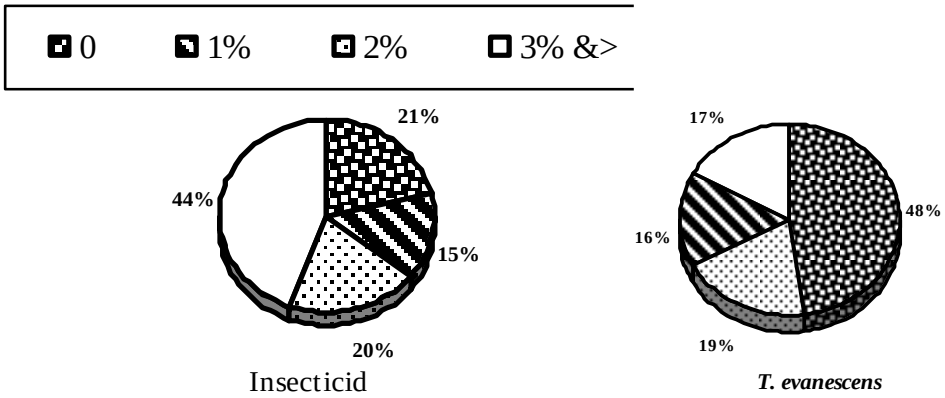

Fig. 4. Categories of the infestation levels of all samples, collected from *Trichogramma* released and insecticide-treated units throughout 2005 cotton season in Dakahlia Governorate.

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المكافحة الحيوية لدودتي اللوز القرنفلية *Pectinophora gossypiella* (Saund.) و الشوكية *Earias insulana* (Boisd.) في حقول القطن بمحافظة الدقهلية بالإطلاق المتزايد لطفيل الترايكوجراما *Trichogramma evanescens* (Westwood)

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أجريت دراسات حقلية لتقييم فعالية إطلاق طفيل الترايكوجراما في حقول القطن بمحافظة الدقهلية- مصر في موسمي ٢٠٠٤ و ٢٠٠٥ وذلك لمكافحة دودتي اللوز القرنفلية والشوكية. وقد أجريت ست إطلاقات بطفيل *Trichogramma evanesces* المحلي في كل موسم بين كل منها ١٠-١٥يوم وفي مساحة ٩٩٩ و ٦٠٠فدان في كل من الموسمين، على التوالي. وتم مقارنة النتائج المتحصل عليها مع نظيرتها في المساحات المعامله بالبرنامج الموصى به لمكافحة ديدان اللوز في مصر. ويشمل هذا البرنامج استخدام المبيدات عند وصول نسبة الإصابة إلى ٣%.

وقد أوضحت النتائج انخفاض في نسبة الإصابة بديدان اللوز في المساحات المعاملة بالترايكوجراما مقارنة بمثيلتها المعاملة بالمبيدات بمقدار ٢٣,٧٣ و ٤٠% حيث سجلت نسبة الإصابة ١,٣٥ و ١,٤٤% في الوحدات المعاملة بالترايكوجراما و ١,٧٧و ٢,٤٠% في الوحدات المعاملة بالمبيدات. وقد نجحت الترايكوجراما في حفظ مستوى الإصابة بديدان اللوز دون الحد الحرج في ٧٨ و ٨٤% من مجموع العينات التي تم فحصها من المساحات التي تم فيها إطلاق الطفيل خلال موسمي التجربة مقابل ٦٧ و ٥٦% في العينات التي تم فحصها من المناطق المعاملة بالمبيدات.