USING *TRICHOGRAMMA EVANESCENS* WESTWOOD FOR CONTROLLING *PECTINOPHORA GOSSYPIELLA* (SAUND.) AND *EARIAS INSULANA* (BOISD.) IN KAFR EL- SHEIKH GOVERNORATE, EGYPT

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INTRODUCTION

Augmentative biological control, where natural enemies are periodically introduced, is commercially applied on large areas in various cropping systems worldwide. Initially augmentative biological control was used to manage pests that had become resistant to pesticides. The use of selective chemicals is an important strategy within pest management programs, since it reduces the population of the phytophagous insects without affecting significantly the natural enemies. In addition, to maximize the compatibleness between the chemical and biological controls, it is needed to know the selectivity and the conditions suitable use of an insecticide, in order to reduce its impact on the natural enemies. Insecticides such as Bt (formulations of *Bacillus thuringiensis*) and some insect growth regulators have very little or no impact on *Trichogramma* and can be used in IPM programs with this egg parasitoid (Denilson *et al* 2003).

In Egypt, the basic studies for mass rearing and released of *Trichogramma* spp. against cotton bollworms began in 1992 at Cotton Bollworms Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza. Biological control in cotton through *Trichogramma* spp. releases has been facilitated by the activities of the same Department since 1995. Between 1995 and 2003, releases of this parasitoid to control cotton bollworms (pink and spiny bollworms) were initiated in cotton fields at Sharkia and Qalyubia Governorates. The use of parasitoids reduced insecticide applications by a significant amount (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). In 2003, Ministry of Agriculture and Land Reclamation established 18 laboratories at different Governorates for producing *Trichogramma* with the aim of controlling cotton bollworms and several lepidopteran pests on vegetables and other

crops. In 2004 cotton season, more support was achieved by the aforementioned Ministry for releasing *Trichogramma* in a large scale in cotton fields. The treated area increased from one thousand feddan at 2003 cotton season to 12000 feddan at 2004 cotton season. This work was conducted at Kafr El- Sheikh Governorate, Egypt in 2003 and 2004 cotton seasons to evaluate a recommended biological control program against pink bollworm, *Pectinophora gossypiella* (Saund.) and spiny bollworm *Earias insulana* (Boisd.). This program includes the local egg parasitoid *T. evanescens* and the recommended formulations of *Bacillus thuringiensis* and some insect growth regulators if this parasitoid fails to maintain boll infestation below 3% (economic threshold level of infested bolls in Egypt).

MATERIAL AND METHODS

Rearing Technique

T. evanescens Westwood was reared on angoumois grain moth, *Sitotroga cerealella* (Oliv.) eggs. For efficient mass rearing of the parasitoid, *S. cerealella* eggs (<24 hr old) were glued to paper cards (21X15 cm.) and exposed to *T. evanescens* adults in glass jars (2-liters capacity) provided with 10% sucrose solution for nutrition and covered with 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.

The Releasing Cards

T. evanescens was released as mature pupa into the field using a release card that protects them from predators and unfavorable weather. The release card prepared in the laboratory from a thick paper (6 x 8cm) folded to make a closed container of 4 x 6 cm, in which a strip of paper that contains grain moth parasitized eggs (about 1000 parasitoids) one day before emergence was put into this container. Twenty-two cotton plants/ feddan were selected to serve as release points. The distance between these points was 14m, and started 7 m from the edges of the field. Cards were hand-placed before the sunset 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.

A sample of 20 cards (from the cards of each release) was maintained at the laboratory under the same rearing conditions to evaluate the quality of the released

parasitoids. In addition, another identical sample of these cards was collected randomly six days after release to estimate the parasitoid emergence under field conditions. The obtained data revealed that more than 90% of the parasitoids succeeded to emerge under both the laboratory and field conditions. Also, high percentage of females (60- 71.5% females) emerged from the laboratory samples.

Field Application

Release of *T. evanescens* was conducted at Kafr El- Sheikh Governorate, Egypt during two successive cotton seasons (2003 and 2004) for controlling pink bollworm, *P. gossypiella* and spiny bollworm *E. insulana*. The cottonseeds (Giza 86) were planted at the two seasons in the control and treated areas. Because this study was conducted in farmers' cotton fields, insecticides were applied for bollworms if samples showed that release of *Trichogramma* failed to maintain pest densities below 3% (economic level of infested bolls in Egypt). Accordingly, formulations of *Bacillus thuringiensis* and some insect growth regulators which have very little or no impact on *Trichogramma* were used.

2003 cotton season. In this season, 12 units (15-33 feddan/ each) were selected in Kafr El- Sheikh Governorate for *Trichogramma* release (9 units) and control (3 units). The total experimental area was 200 feddan for *Trichogramma* release and 84 feddan for control. Eight *Trichogramma* releases were applied at about 10 days intervals (June 21st - September 2nd), while, the recommended bollworms insecticide control program was applied at the control units. This program used 4 chemical insecticide applications (organophosphoric and pyrethroid compounds) through this season at 13-15 days intervals.

2004 cotton season. *Trichogramma* released area increased to 784.25 feddan, distributed into nine regions (2-4 units/ region) namely Kafr El-Sheikh (100 feddan), Dosouk (105 feddan), Killeen (52 feddan), Fowah (86 feddan), Matopus (100 feddan), Seedy Salem (90 feddan), Peyla (82 feddan), Al-Hamole (112.75 feddan) and Al-Riyadh (56.5 feddan). Six releases with *T. evanescens* were applied at 10 days intervals (from July 6th until September 9th). As for control, 3-4 units (15-20 feddan/ each) were selected from each of the aforementioned regions and were treated with the recommended chemical insecticides as the previous season.

At the two seasons, the release area was separated by 5 feddan (at least) from the control area to avoid dispersal of released *Trichogramma* into control plots. The sequential sampling method was used to evaluate the infestation of green cotton bolls at the two seasons. Therefore, boll samples (100 green bolls/ each) were

collected randomly from *Trichogramma* and control treatments at 6 days intervals, dissected and the number of infested bolls was recorded. The 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 or more infested bolls. Therefore, the area that received bollworms densities of 3% or more (the fourth category) was treated with formulation of insect growth regulators (consult or atabron). Analyses of variance (ANOVA) between *Trichogramma* and insecticides treatments were conducted on data of the two seasons.

RESULTS AND DISCUSSION

2003 cotton season. In this season, releasing of *Trichogramma* was timed to cotton flower appearance. The first three releases of *Trichogramma* were done at June 21st, July 1st and 12th, respectively, while the first green cotton boll samples were available at July 15th. Few numbers of green cotton bolls were infested with spiny bollworm throughout the whole season comparing with pink bollworm. Accordingly, the number of infested bolls with the two pests was recorded as a total. Table (1) shows the mean percentages of the total infestation (pink and spiny bollworms) in the sequential samples. In the first inspection, the three samples (100 bolls/ sample) from the area treated with chemical insecticides (control) were free from infestation, while 2 bolls from the 10 samples collected from *Trichogramma* treated area, were infested (infestation averaged 0.2%). In the second inspection (July 21st), 3 infested bolls were recorded from *Trichogramma* treated units (12 samples) to give 0.25% infestation opposed to 0.67% in control area. The 4th release of *Trichogramma* was done at July 22nd. In the following inspection, an obvious increase in the boll infestation occurred in the control (insecticide) samples as it averaged 2.33%, while the infestation was stable at *Trichogramma* release units (0.25%). The 5th release of *Trichogramma* was done at August 2nd. Percentage of infested bolls in *Trichogramma* units increased to 1.67 and 1.5% at the 4th and 5th inspections, respectively.

On the contrary, slight decrease in infestation occurred in the control unites as it averaged 2% at the two inspections. On August 14th, the percent of infestation averaged 1.92 and 1.33% in *Trichogramma* and control units, respectively. Another increase in infestation occurred in *Trichogramma* units to reach 2.75% at the 7th inspection, while it decreased to 0.33% in control (insecticide treatments). The 7th *Trichogramma* release was applied at August 23rd. At the following two inspections, percentage of infested bolls

decreased to 1.33 and 0.83% in *Trichogramma* units, opposed to 0.67 and 1.33% in control units, respectively. The last *Trichogramma* release occurred at September 2nd, while the last inspection was at September 7th. At this time, the percent of infested bolls averaged 1.67 and 1.0% in *Trichogramma* and insecticide treatments, respectively. At the end of the season, the estimated whole percentage of infestation averaged 1.25% in *Trichogramma* release units opposed to 1.17% in insecticide units.

TABLE (I)

Infestation of green cotton bolls with pink and spiny bollworms (as a total) in *T. evanescens* released units comparing with insecticide-treated units at Kafr El-Sheikh Governorate through 2003 cotton season

Inspection	Date	Infestation (%)	
		T. evanescens units	Insecticides units
1st	July 15th	0.20	0
2nd	July 21st	0.25	0.67
3rd	July 27th	0.25	2.33
4th	Aug. 2nd	1.67	2.00
5th	Aug. 8th	1.50	2.00
6th	Aug. 14th	1.92	1.33
7th	Aug. 20th	2.75	0.33
8th	Aug. 26th	1.33	0.67
9th	Sept. 1st	0.83	1.33
10th	Sept. 7th	1.67	1.00
Mean		**1.25**	**1.17**

ANOVA yielded no significant difference between the total percentages of infestation with bollworms in *T. evanescens* and insecticide treatments (F= 0.0395)

Regarding the green boll infestation throughout the 10 inspections (Table 1), it noted lower infestation in *Trichogramma* samples at the first five inspections than in insecticide samples (control). The opposite was true at the following three inspections (6th, 7th and 8th inspections). This increase may be due to releasing insufficient numbers of parasitoids that were disable 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.

Regarding the infestation levels in all the dissected green boll samples in Fig. (1), it could be noted that 46% of samples from both *Trichogramma* (118 samples) and insecticide (30 samples) units were free from any infestation. Meanwhile 24 & 20%

and 9 & 17% of these samples have 1 and 2 infested bolls, respectively. Thus, *T. evanescens* succeeded to maintain pink and spiny bollworm densities below the economic level of infestation (3%) in 79% of these samples, since 21% of the samples have 3 and more infested bolls. According to Ministry of Agriculture Control Program, treatment with insect growth regulator (consult or atabron) was done in units, which their boll samples reached or exceeded the economic level of infestation (3%). As for control, the four applications with chemical insecticides were able to maintain these densities below the economic level of infestation in 83% of the dissected samples, while 17 % 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 = 0.0395).

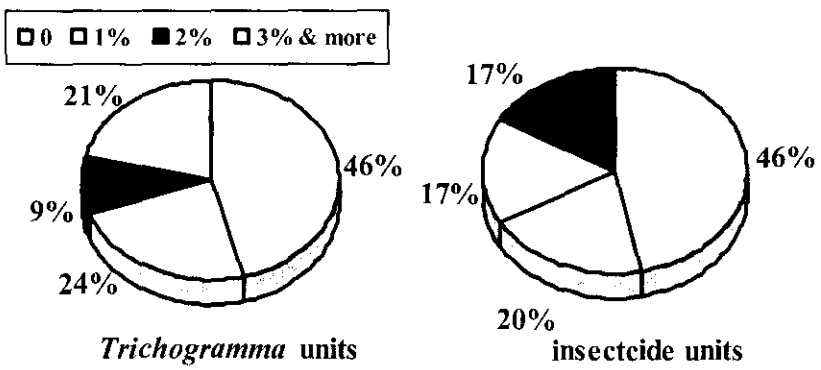

Fig.1. Categories of the infestation levels of all samples, collected from *Trichogramma* released and insecticide-treated units throughout 2003 cotton season in Kafr El-Sheikh Governorate.

Data in Table (2) reveal the mean seasonal infestation in the different units (9 units) of *Trichogramma* released and insecticide treated units (3 units). These units showed different levels of infestation. The lowest percentage of infestation (0.6%) was recorded in the unit number 208 followed by unit number 43 (0.74%). On the contrary, the highest percentage of infestation (2.4%) was recorded in unit number 218. The remaining units showed averages of infestations ranged between 1.0 – 1.74%. As for insecticide treatments, infestation averaged 0.9, 1.2 and 1.4 in the units' number 44, 209 and 219, respectively.

TABLE (II)

Infestation of green cotton bolls with pink and spiny bollworms (as a total) in different units treated with *T. evanescens* comparing with others treated with insecticides at Kafr El-Sheikh Governorate (2003 cotton season)

The ID of the treated unit (replicates)	Area (Feddan)	No. Samples (100 bolls/ sample)	No. infested bolls	% Infestation
		T. evanescens		
37	16	10	12	1.20
38	15	10	10	1.00
39	21	20	25	1.25
43	24	19	14	0.74
50	24	19	33	1.74
201	19	10	14	1.40
208	22	10	6	0.60
218	33	10	24	2.40
220	25	10	10	1.00
Total	200	118	148	
Mean		**13.11**	**16.44**	**1.25**
		Insecticides		
44	25	10	9	0.90
209	30	10	12	1.20
219	29	10	14	1.40
Total	84	30	35	
Mean		**10**	**11.67**	**1.17**

2004 cotton season. In this season, *T. evanescens* was released for 6 times (July 6th - September 9th) in 784.25 feddan distributed at 9 regions. Table (3) shows the percentages of boll infestation in all inspections throughout the whole season (12 inspections, 100 bolls/ inspection). These percentages ranged between 0.43- 2.00% in *Trichogramma* treatments and 0.54- 1.56% in insecticide treatments. Statistical analysis of infestation in the different inspections showed no significant difference between *Trichogramma* and insecticide treatments (F value = 0.1634).

Regarding the infestation levels in all the dissected green boll samples in Fig. (2), it could be noted that 65% of samples from *Trichogramma* treated units were free from any infestation opposed to 51% in samples from insecticide treated units. Meanwhile, 10 & 15% and 7 & 8% of the samples from the two treatments have 1 and 2 infested bolls, respectively. Thus, *T. evanescens* succeeded to maintain pink and spiny bollworm densities below the economic level of infestation in 82% of

these samples (Fig., 2), since 18% of the whole samples had 3 or more infested bolls. Accordingly, treatment with insect growth regulators (consult or atabron) was applied in these areas. As for insecticide treatments, the four applications with chemical insecticides were able to maintain these densities below the economic level of infestation in 74% of the dissected samples only, while 26% of the whole samples had 3 and more infested bolls.

TABLE (III)

Infestation of green cotton bolls with pink and spiny bollworms (as a total) in *T. evanescens* released units comparing with insecticide-treated units throughout 2004 cotton season in Kafr El-Sheikh Governorate

Inspection	Date	Infestation (%)	
		T. evanescens	Insecticides
1st	July 12th	0.43	0.54
2nd	July 18th	1.12	0.97
3rd	July 24th	1.31	1.22
4th	July 30th	0.79	1.20
5th	Aug. 5th	1.69	1.41
6th	Aug. 11th	2.00	1.52
7th	Aug. 17th	1.24	1.41
8th	Aug. 23rd	1.27	1.27
9th	Aug. 29th	1.03	1.56
10th	Sept. 4th	1.10	1.03
11th	Sept. 10th	1.43	1.12
12th	Sept. 16th	1.14	0.9
Mean		**1.22**	**1.18**

ANOVA yielded no significant difference between the total percentages of infestation with bollworms in *T. evanescens* and insecticides treatments (F= 0.1634).

The number of samples from each region differed according to the regions' area. The total number reached 335 and 408 samples (100 bolls/ sample) from *Trichogramma* released and insecticide-treated regions, respectively. While the total number of infested bolls reached 410 & 482 bolls, indicating 1.22 and 1.18% infestation in the two treatments through the whole season, respectively (Table, 4). Regarding the mean percentage of infestation at the different regions in the same Table, lower percentages of infestation with spiny bollworm could be noted comparing with pink bollworm. In *Trichogramma* regions, the percentages of infestation with the two pests ranged between 0.04- 0.25% and 0.85- 1.53%, respectively; while they ranged between 0.07- 0.32% and 0.73-1.3% in insecticide treated regions, respectively. In general, the total infestation with the two insects ranged between 0.94- 1.65% and 1.03- 1.44% in the aforementioned treatments,

respectively. Statistically, there is no significant difference in region infestations between *Trichogramma* and insecticide treatments (F value = 0.5174).

In the present study, good results were obtained by using a control program included *T. evanescens* and environmentally safe pesticides (Bt and IGRs) when it is necessary. Another program was used in India by Tuhan *et al.* (1987), as they released *Trichogramma brasilience* 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. The increase in seed cotton was 90-Kg ha-1. 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*. In Egypt, these results were in agreement with our previous results when the local or imported species of *Trichogramma* were released in cotton fields (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).

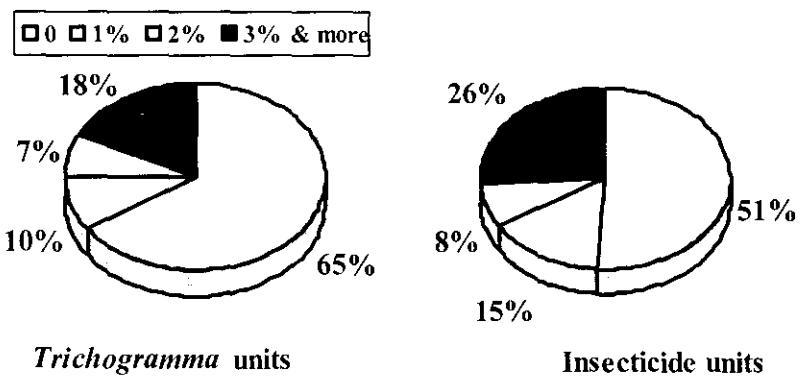

Fig. 2. Categories of the infestation levels of all samples, collected from *Trichogramma* released and insecticide-treated units throughout 2004 cotton season in Kafr El-Sheikh Governorate.

TABLE (IV)

Infestation of green cotton bolls with pink bollworm (PBW) and spiny bollworm (SBW) in *T. evanescens* released units comparing with insecticide-treated units in Kafr El-Sheikh Governorate (2004 cotton season)

Region	*T. evanescens*					Insecticides				
	No. Samples	No. Infested bolls	Infestation (%)			No. Samples	No. Infested bolls	Infestation (%)		
			PBW	SBW	Total			PBW	SBW	Total
Kafr El-Sheikh	48	53	0.85	0.25	1.10	44	49	0.95	0.16	1.11
Dosouk	46	43	0.85	0.09	0.94	44	51	1.09	0.07	1.16
Killeen	24	35	1.42	0.04	1.46	48	61	1.17	0.10	1.27
Fowah	36	59	1.53	0.11	1.64	44	63	1.30	0.14	1.44
Matopus	42	53	1.02	0.24	1.26	40	49	0.95	0.28	1.23
Seedy Salem	36	45	1.06	0.19	1.25	48	60	0.98	0.27	1.25
Peyla	33	40	1.03	0.18	1.21	44	45	0.73	0.30	1.03
Al-Hamole	46	51	1.00	0.11	1.11	48	52	0.98	0.10	1.08
Al-Riyadh	24	31	1.08	0.21	1.29	48	52	0.79	0.32	1.18
Total	**335**	**410**	**1.06**	**0.16**	**1.22**	**408**	**482**	**0.99**	**0.19**	**1.18**

ANOVA yielded no significant difference between the total percentages of infestation with bollworms in *T. evanescens* and insecticides treatments (F= 0.5174).

According to the present results, the use of *T. evanescens* to control *P. gossypiella* and *E. insulana* in cotton fields is biologically possible. However, this requires increase awareness among farmers to encourage them using this parasitoid. In addition, development of a release strategy to optimize field efficiency is necessary. This strategy includes: 1). The first release of *Trichogramma* in cotton fields should coincide with the appearance of the first cotton fruit branch (55-60 days after planting). 2) The release card must contain three ages of the parasitoid to emerge in waves and cover a control period of 10 days in the field. 3) Additional releases should be made at 10 days intervals. 4) The number of released parasitoids should be increased according to the increase of the target pest. 5) The economic level of infestation should be increased to 5% at least to avoid the use of insecticides and to allow the build up of the released parasitoids and natural enemies in the field.

SUMMARY

Field study was conducted at Kafr El- Sheikh Governorate, Egypt in 2003 and 2004 cotton seasons to evaluate a recommended biological control program against pink bollworm, *Pectinophora gossypiella* (Saund.) and spiny bollworms

Earias insulana (Boisd.). This program includes the local egg parasitoid *T. evanescens* and use of recommended formulations of *Bacillus thuringiensis* and some insect growth regulators if this parasitoid fails to maintain boll infestation below 3% (economic level of infested bolls in Egypt). The total area for *T. evanescens* release was 200 and 784.25 feddan at 2003 and 2004 cotton seasons, respectively. While another area was selected as control. The release area was separated by 5 feddan at least from the control area to avoid dispersal of released *Trichogramma* into control plots. Eight and six *Trichogramma* releases were applied at the two seasons, respectively. While, the recommended bollworms insecticide control program was applied at the control units. This program used 4 chemical insecticide applications (organophosphoric and pyrethroid compounds) in the two seasons. The obtained results indicated that *T. evanescens* succeeded to maintain boll infestation below the economic level in 79 and 82% of the dissected boll samples in the two seasons, respectively. As for the control, the four applications with chemical insecticides were able to maintain this boll infestation below the economic level in 83 and 74% of the sampled bolls in the two seasons, respectively. Statistically, there is no significant difference at the two seasons between the infestation post treatment with *Trichogramma* or with insecticides.

REFERENCES

ABD EL-HAFEZ, A. (2004): Evaluation of four trichogrammatids as biological control agents for *Pectinophora gossypiella* (Saund.) in Egypt. *(Beltwide Cotton Conferences, Cotton Insect research and Control Conference, San Antonio, TX January 5-9, 1458-1462).*

ABD EL-HAFEZ, A. and M. A. NADA (2000): Augmentation of *Trichogrammatoidea bactrae* Nagaraja in the IPM programs for control of pink bollworm, *Pectinophora gossypiella* (Saund.) in Egypt. *(2000 Beltwide Cotton conferences, Cotton Insect research and Control Conference, 1009-1014).*

ABD EL-HAFEZ, A.; F.F. SHALABY; E.F. EL-KHAYAT and M.A.A. EL-SHARKAWY (2002): Efficiency of late season releasing of four trichogrammatid species in suppressing infestation with *Pectinophora gossypiella* (Saund.) in cotton fields at Sharkia Governorate. *(2nd International Conference, Plant Protection Research Institute, Cairo, Egypt, 21-24 Dec., 1: 605-610).*

ABD EL-HAFEZ, A.; M. A. EISSA; M. A. NADA and M.F. GERGIS (2004): Effectiveness of *Trichogramma evanescens* for controlling pink bollworm, *Pectinophora gossypiella* (Saund.) in cotton. *(Beltwide Cotton Conferences, San Antonio, cotton Insect research and Control Conference, TX January 5-9, 1463-1469).*

CHAO-YUZHI; LIU-JINHUA; YZ-CHAO and JH-LIU (1996): The induction of adversity-resistant *Trichogramma flavum* and its use in the experimental control of bollworms. *(China-Cottons, (10): 23-26).*

DENILSON, B. C.; S. BRIGIDA; A. C. GERALDO and F. C. CESAR (2003): Residual action of insecticides to larvae of *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) under greenhouse conditions. *(Ciênc. Agrotec. Lavras., 27(4): 835-839).*

SHALABY, F.F.; A. ABD EL-HAFEZ; E.F. EL-KHAYAT and M. A.A. EL-SHARKAWY (2002): Applied control of *Pectinophora gossypiella* (Saund.) and *Earias insulana* (Boisd.) in cotton fields by releasing *Trichogrammatoidea bactrae* Nagaraja. *(2nd International Conference, Plant Protection Research Institute, Cairo, Egypt, 21-24 Dec., 1: 402-411).*

TUHAN, N. C.; A. D. PAWAR and R. S. ARORA (1987): Use of *Trichogramma brasiliensis* Ashmead against cotton bollworms in Srigangangar, Rajasthan, India. *(J. Adv. Zool. 8: 131-134).*