

Applications of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) and *Bacillus thuringiensis* for Controlling the European Corn Borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae),

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ABSTRACT

Laboratory and field studies to evaluate the efficiency of biological control agents against one of the main pests, infesting maize plants, the European corn borer, *Ostrinia nubilalis* Hübner, using inundative releases of the egg parasitoid, *Trichogramma evanescens* Westwood alone or integrated with *Bacillus thuringiensis* (*B.t.*) application were conducted, at Sakha Research Station, Kafr El-Sheikh Governorate for three successive maize seasons 2001, 2002 and 2003. *B.t.* applications targeted the larvae escaped from *T. evanescens* parasitism during the egg stage. Effect of *B.t.* on different developmental stages of *T. evanescens* inside the host eggs was tested under laboratory conditions. A slight reduction of the adult parasitoid emergence was recorded. Based on the three seasons' data, the mean natural parasitization rates on *O. nubilalis* eggs by *Trichogramma* in the experimental area were 38.37, 36.00 and 36.82% for 2001, 2002 and 2003, respectively. After two releases, with a total of 96000 parasitoids/feddan, each year, the mean rates of parasitization reached 74.72, 76.83 and 77.23% in the plots treated with the parasitoid alone and 72.90, 74.21 and 75.56% in the plots treated with *Trichogramma* plus *B.t.*, respectively. Reduction percentages of *O. nubilalis* population reached 51.79 and 73.21% in 2001, 52.38 and 73.81% in 2002 and 56.00 and 76.00% in 2003, in the parasitoid released plots alone and in those integrated with *B.t.* Also, the percentage of infested nodes, average numbers of pores and the damaged numbers of ears and tassels in the plots treated with *Trichogramma* combined with *B.t.* were dropped significantly as compared to the control.

Key Words: *Trichogramma evanescens*, *Bacillus thuringiensis*, *Ostrinia nubilalis*, Field Application

INTRODUCTION

Maize (*Zea mays* (L.)) is one of the major cereal crops worldwide. In Egypt, maize is the staple food for the majority of farmers; its foliage and grains is a main constituent in cattle food. The European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), is one of the most injurious maize pests in Kafr El-Sekh region. The full-grown larvae of *O. nubilalis* are diapausing, within crop residues during winter then pupate and emerge in the spring. Adults lay egg masses on the corn leaves, early instars feed on the leaf around the egg mass and later within the whorl, then bore into the stalk behind the leaf sheath, usually at the node causing damage in the developing tassels, ears, and midribs. Moreover, under the severe attack, stalks and tassels are broken (El-Naggar, 1991). Its first egg clusters usually occur 45 days after sowing of maize seeds (Awadallah, 1974 and Allam, 2003).

Integrated Pest Management (IPM) involves the use of many techniques, including biological control, to provide effective control of crop pests, with minimum use of pesticides. The application of chemicals has disrupted the effectiveness of the parasitoids, and also greatly reduced population of predators. So, using the egg parasitoid *Trichogramma* spp. instead of insecticides, preserves the endemic natural enemy complex, reduces the need for additional treatments to control secondary pests and reduces the risks to human and environmental health that are associated with insecticides (Pimentel *et al.*, 1993 and Smith, 1996). Economically, *Trichogramma* can be used cost-effectively in high-value crops, such as seed corn and possibly sweet corn (Andow, 1997).

The species, *T. evanescens* was successfully used in several countries such as, the former USSR (Beglyarov and Smitnik, 1977) and Germany (Hassan, 1993) for controlling *O. nubilalis*, in maize fields. The combination of parasitoids, predators and applied pathogens provided good control for certain maize pests where it appears that *B.t.* toxins are not as harmful as the majority of insecticides (Hassan *et al.*, 1987 and Takada *et al.*, 2001).

The aim of the study was to test the susceptibility of *T. evanescens* developmental stages to *B.t.* in laboratory and to evaluate the field releases of the egg parasitoid separately or integrated with *B.t.* applications against *O. nubilalis* in maize fields.

MATERIALS AND METHODS

Rearing techniques:

- The European corn borer, *O. nubilalis* was reared, following the technique of Abdel Samea (1990), in a controlled room under constant temperature of $27 \pm 1^\circ\text{C}$ and $60 \pm 10\%\text{R.H.}$
- The egg parasitoid, *T. evanescens*, was maintained on the eggs of *Sitotroga cerealella* (Olivier) as described by Abbas (1998) under the same conditions.

Both *O. nubilalis* and *T. evanescens* starters were collected from the maize fields at Sakha Research Station.

Bioinsecticide (Agerin): The commercial bioinsecticide Agerin (32000 I.U./mg) of *B.t.* subsp. *aegypti* produced under the license from (AGERIN) by Biogro International, Egypt. Recommended rate of application was 250g/ feddan.

Laboratory tests: Five egg clusters of *O. nubilalis*, less than 24h old, were exposed to 20 mated female wasps in a glass tube for 30 minutes. The parasitized eggs were incubated at $26 \pm 1^\circ\text{C}$ for 1, 3, 5 and 7 days, then dipped for 10 seconds in Agerin recommended concentration (250g/ feddan). Control was dipped in distilled water. Each treatment was performed seven times. The treated host egg masses were kept at $26 \pm 1^\circ\text{C}$ until emergence of parasitoid adults. Percentage of parasitoid emergence inside the host was calculated.

Field experiments: The study was conducted at Sakha Research Station, (Kafr El-Sheikh Governorate) during the maize seasons of 2001, 2002 and 2003. The maize variety "Giza 2" seeds were used. In each season, an area of about half feddan (2000 m²) was chosen and divided into 12 equal plots; each had 10 rows with 25 hills/row. Four treatments with three replicates were arranged in a complete randomized design. The four treatments were: untreated control, the bioinsecticide (Agerin) alone, and release of *T. evanescens* and the integration of Agerin plus *T. evanescens* applications. The unreleased plots (control and Agerin application plots) were separated by a minimum of 300 m from the other *Trichogramma* releasing plots to reduce possibility of parasitoid dispersal to them.

Agerin application: It was applied by knapsack sprayer (20L.) and using a total volume of 200 L/feddan. It was applied two times in weekly interval, on 29th August and 5th September 2001 and 2003, while respective applications in 2002 were on 27th August and 3rd September. In the integrated plot of *Trichogramma* and *B.t.*, *B.t.* was applied always before the parasitoid release, except the first application on season, 2003 which was carried out 4 days after *Trichogramma* release.

Trichogramma releases: Parasitized eggs were affixed on cards kept inside carton envelopes to help parasitoids' emergence and to protect them from direct sunshine and predators. Releases of *T. evanescens* began 45 days after sowing for two times, bi-weekly interval, on 29th August and 12th September 2001, on 27th August and 10th September, 2002 and on 24th August and 12th September 2003. A total of 96000 parasitoids / feddan (2000 parasitoids/ plot) were released every season.

Field sampling: All plots were sampled weekly during the experimental period. *O. nubilalis* egg batches were collected randomly from maize plants. Egg samples were transferred to the laboratory, the number of parasitized eggs, number of hatched eggs and dead ones were determined. Hatched larvae from unparasitized eggs were removed from the cups, because they might attack the unhatched and parasitized eggs.

Harvest data: To evaluate the impact of different treatments on the yield components by harvest time, numbers of infested nodes, pores, ears, tassels and weight of 10 ears were estimated from ten randomly sampled plants per each plot. Also, numbers of *O. nubilalis* larvae were estimated from each collected maize plant.

Statistical analysis: Egg parasitism rates was estimated by the number of eggs that yielded *T. evanescens* adults divided by the total number of eggs collected (i.e., dead eggs were included). Egg parasitism rates and other studying yield parameters were compared in the unreleased and released plots, using repeated measures ANOVA. Mean values were separated by the least significant difference (L.S.D.) at $P = 5\%$ (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Laboratory tests:

The sensitivity of various developmental stages of *T. evanescens* to *B.t.* treatment was studied to determine the best timing for releasing the parasitoid wasps and *B.t.* application under field conditions. The percent reduction of the wasps' emergence was 2.33, 1.52, 1.49 and 1.71% for *O. nubilalis* eggs treated with *B.t.* on the 1st, 3rd, 5th and 7th day after parasitization, respectively. Results in fig. (1), indicate that *B.t.* application differed in its effect on adults' emergence depending upon the intervals between date of host egg parasitisation and *B.t.* application. The bioinsecticide (Agerin) had a negligible effect on parasitoid's emergence. The adverse effect of *B.t.* decreased on the 3rd and 5th days after parasitism. No significant reduction was observed in the emergence rate of the treated *O. nubilalis* parasitized eggs as compared to control of each parasitoid age. Borah and Basit (1996) found that the emergence of *Trichogramma japonicum* from eggs of *Corcyra cephalonica* treated with *B.t.* on the 3rd or 6th days after parasitism had a little effect on emergence of parasitoids. Adults' emergence was relatively lower when eggs were sprayed on the 6th day after parasitism compared with those sprayed on the 3rd day after parasitism. Radhika (1998) reported that *B.t.* had a negligible effect on duration of the egg, larval and pupal stages, and adult emergence of *Trichogramma* spp. reared on eggs of *C. cephalonica*.

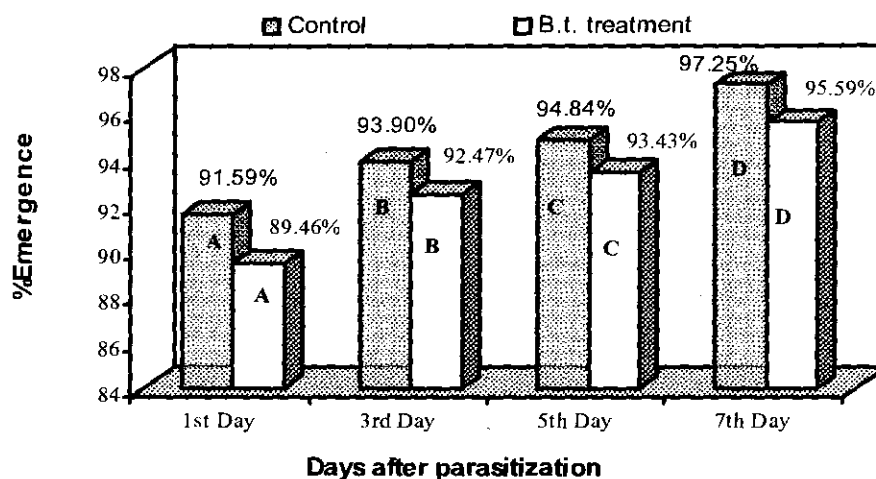


Fig. (1): Percent emergence of parasitized eggs after laboratory treatment. Similar alphabetical letters in columns indicate insignificant difference between each two columns (T-test).

Field trials:

Egg Parasitism:

The role of parasitism in reducing the survivorship of egg masses was evaluated in both; the *T. evanescens* released plots alone or integrated with *B.t.* for controlling *O. nubilalis* as compared with the control plots of *B.t.* treatment. Rate of parasitism in the unreleased plots, during the three years of experiments (2001-2003), began in August, peaked in September and declined sharply in October. The peak of *O. nubilalis* egg populations in the maize field at Kafr El-Sheikh region usually occurs during September (Zanaty and Shenishen, 1989). Overall means of parasitization rates of natural egg masses sampled in the three years were 38.37 and 39.23%; 36.00 and 39.61% and 36.82 and 36.00% for control and *B.t.* treated plots, respectively. These rates were obviously higher in the release plots where they reached 74.72 and 72.90 %; 76.83 and 74.21% and 77.24 and 75.56% in *T. evanescens* alone and *B.t.* + *T. evanescens* plots, respectively, (Table, 1 and Fig., 2).

Weekly rates of parasitism in the treated unreleased and released maize plots during the seasons 2001, 2002 and 2003 are illustrated in (Fig., 3). Generally, the highest rate in the control plots, through the three seasons, reached 90.91 and 83.33% on 26th Sept. and 2nd Oct. 2003, while being 88.00% on 26th Sept. of the same year in the unreleased *B.t.* treated plots. In the releasing plots, parasitization rate reached 100% around the end of the seasons, in almost all the years. Statistically, the release plots had significantly higher rates of parasitism than the control and *B.t.* treated plots during the three seasons of the experiment. Also, there was no significant difference in the results of parasitism rates between the two release plots (with or without *B.t.* applications).

Table (1): Overall percent of parasitism on the European corn borer, *O. nubilalis* egg clusters in the released and unreleased plots at Kafr El-Sheikh Governorate during the seasons of 2001, 2002 and 2003.

Treatments	Sample Collected	2001					2002					2003				
		Aug.	Sept.	Oct.	Overall	% Parasitism	Aug.	Sept.	Oct.	Overall	% Parasitism	Aug.	Sept.	Oct.	Overall	% Parasitism
Control	A	13	238	7	258	38.37	8	242	0	250	36.00	14	238	6	258	36.82
	B	2	92	5	99		1	89	0	90		1	89	5	95	
<i>B.t.</i>	A	10	236	14	260	39.23	10	245	0	255	39.61	13	237	0	250	36.00
	B	1	96	5	102		1	100	0	101		1	89	0	90	
<i>T.evanescens</i>	A	9	242	14	265	74.72	10	248	1	259	76.83	12	232	6	250	77.24
	B	1	183	14	198		1	197	1	199		4	197	6	207	
<i>B.t. + T.evanescens</i>	A	14	236	12	262	72.90	10	240	2	252	74.21	7	240	3	250	75.56
	B	1	178	12	191		1	184	2	187		3	178	3	184	

1st spray (Aug.29, 2001; Aug.27, 2002& Aug.29,2003)

1st release (Aug.29, 2001; Aug.27, 2002& Aug.24,2003)

2nd spray (Sept.5, 2001; Sept.3, 2002& Sept.5,2003)

2nd release (Sept.12,2001; Sept.10, 2002& Sept.13,2003)

A = Number of collected egg clusters

B = Number of parasitized egg clusters

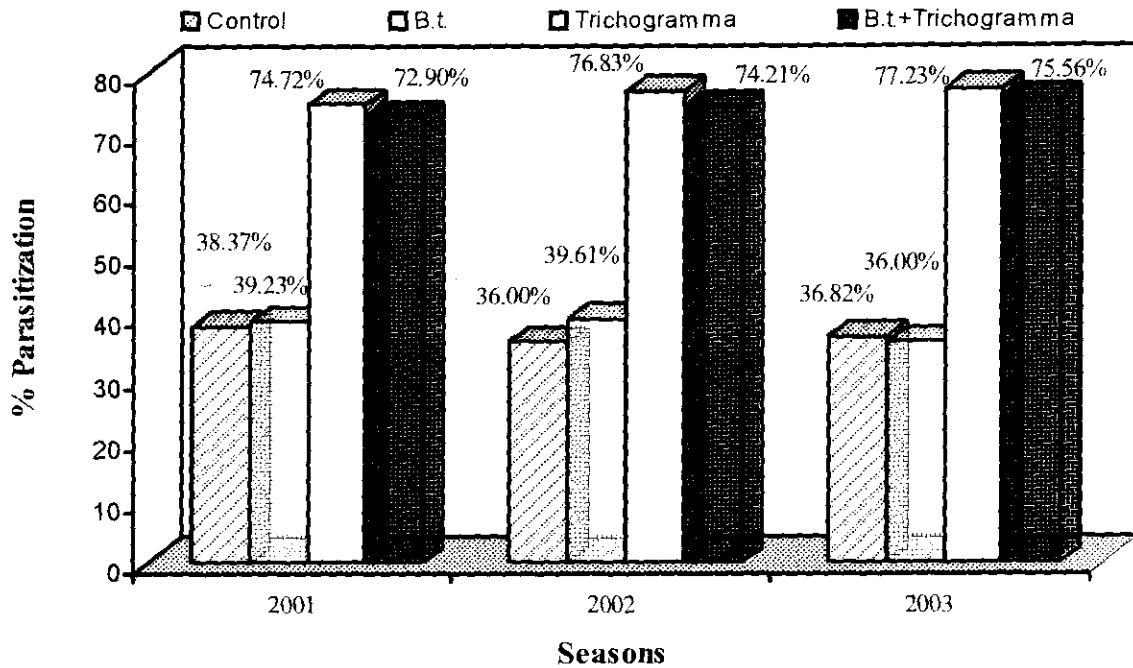


Fig. (2): Percentages of parasitized egg clusters of *O. nubilalis* in proportion to the total collected clusters after different treatments, at Kafr El-Sheikh Governorate during the maize seasons of 2001, 2002 and 2003.

Burgio and Maini (1995) showed that the difference in the egg masses in release plots were highly significant as compared to parasitism in the control. Cagán *et al.*, (1998) stated that the highest natural parasitism on *O. nubilalis* eggs could be found at the end of the egg laying periods according to the influence of the weather conditions. Hot and dry weathers affect the development of the parasitoid.

Crop damage and harvest data:

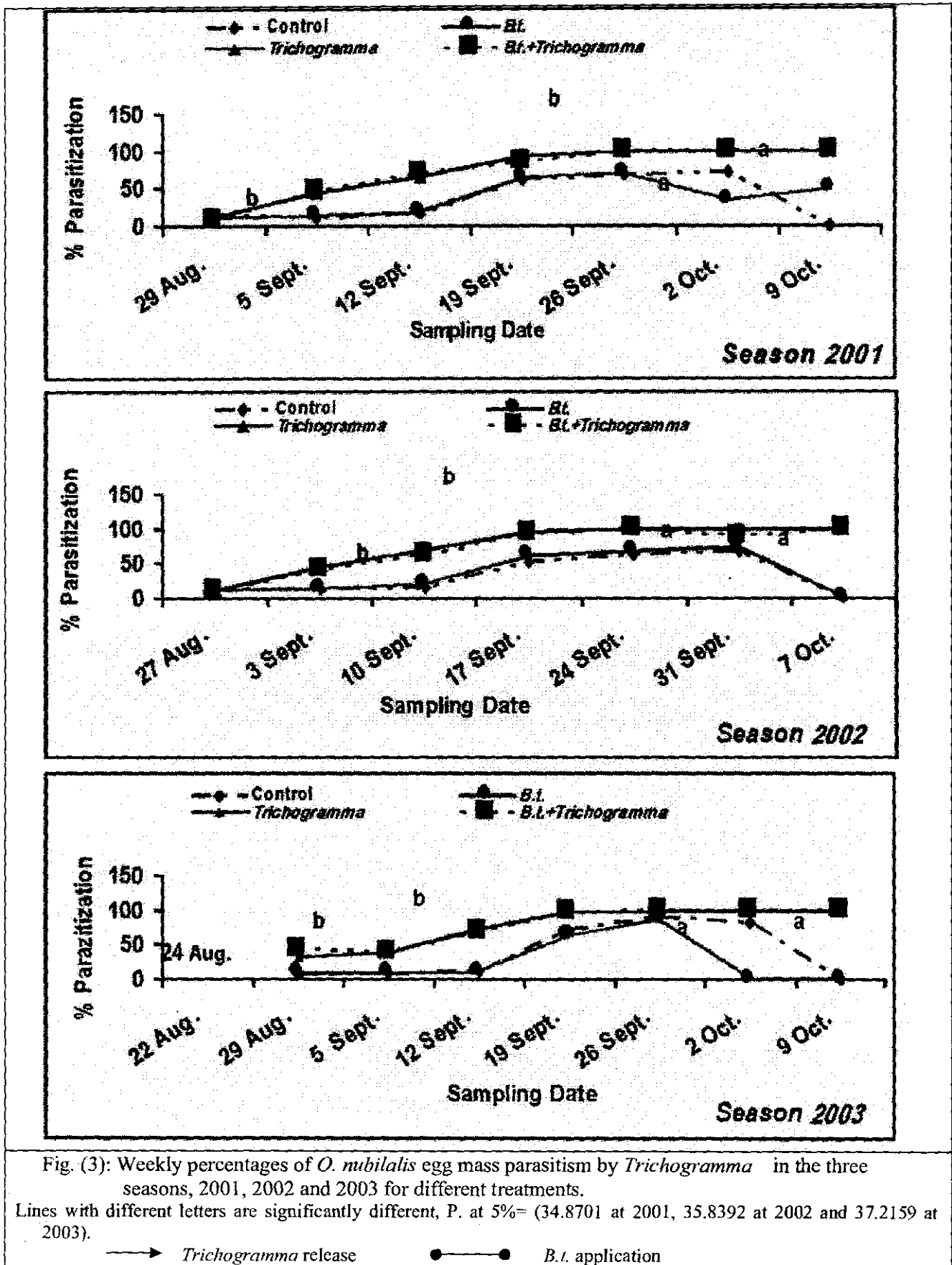
In 2001, the average number of *O. nubilalis* larvae (from ten randomly sampled plants per each plot) ranged between 5.00-18.67, while in 2002 and 2003, it ranged between 3.60-14.00 and 4.00-16.67 for *T. evanescens* + *B.t.* and control plots, respectively. In the three seasons, the integrated effect of *T. evanescens* releases with *B.t.* applications caused significantly more suppression in *O. nubilalis* population compared to the control, *B.t.* and *T. evanescens* treated plots alone, (Table, 2).

Correspondingly, in 2001, the reduction percentage in infested nodes were 15.81, 27.80 and 55.81% compared to 26.19, 33.86 and 56.68% and 28.19, 38.45 and 57.71% in 2002 and 2003 for *B.t.*, *T. evanescens* and *T. evanescens* + *B.t.* treated plots, respectively. Numbers of pores, damaged ears and tassels/ 10 plants by the European corn borer averaged 21.33, 3.00 and 11.33; 30.67, 3.33 and 6.00; 27.67, 3.00 and 5.67 in the control plots in seasons 2001, 2002 and 2003, respectively. The percentage of infested nodes, the number pores and damaged tassels showed insignificant variations between *T. evanescens* and *B.t.* in separately treated plots. While respective parameters in the integrated *T. evanescens* release plots with *B.t.* showed a significant reduction in comparison with the release and control plots. Numbers of damaged ears in *T. evanescens* release plots + *B.t.* were reduced insignificantly than in the control plots and reached 55.56, 60.00 and 66.67 %, during the three seasons, respectively, (Table, 2). In 2003, the efficiency was higher; this is may be due to the timing of parasitoid releases, as it was released four days before *B.t.* application.

The percentages of increase in ears' weight than control reached 1.2768, 1.478 and 3.9369% in 2001; 0.2959, 4.1420 and 4.9290% in 2002 and 1.1030, 1.4725 and 2.5754% in 2003 for *B.t.*, *T. evanescens* and *B.t.* + *T. evanescens* plots, respectively. Ears' weight did not differ significantly among the *T. evanescens* release, *B.t.* treatment and the control. The integration of *B.t.* and *T. evanescens* did not result in additional significant increase in corn ears' weights as compared to the control, *B.t.* and *T. evanescens* treated alone in the three seasons.

Table (2) Percentages of reduction of pores, infested nodes, damaged ears& tassels; ears weight and average numbers of *O. nubilalis* larvae and other corn borers in different treatments/ 10 maize at Kafr El-Sheikh Governorate, seasons 2001, 2002 and 2003.

Treatments	%of Infested Nodes	% Reduction	L. S. D. 5%	Aver. No. of Pores	% Reduction	L. S. D. 5%	Aver. No. of Infested Ears	% Reduction	L. S. D. 5%	Ears Weight/ Kg.	% Increase in Ears Weight	Aver. No. of Infested Tassels	% Reduction	L. S. D. 5%	Aver No.of <i>O. nubilalis</i> Population	% Reduction	L. S. D. 5%
In 2001																	
Control	12.54 a		2.2271	21.33 a		3.2612	3.00		---	1.6917	---	11.33a		2.6627	18.67a		3.9570
<i>B.t.</i>	10.56 ab	15.8		16.33 b	23.44		2.67	11.11		1.7133	0.0700	8.33 b	26.47		11.33b	39.29	
<i>T. evanescens</i>	9.06 b	27.80		14.67 b	31.25		2.33	22.22		1.7167	0.0386	7.00b	38.24		9.00 b	51.79	
<i>T. evanescens</i> + <i>B.t.</i>	5.54 c	55.8		9.00 c	57.81		1.33	55.56		1.7583	3.8604	3.33 c	70.59		5.00 c	73.21	
In 2002																	
Control	11.17 a		2.0287	30.67 a		6.7669	3.33		---	1.6900	---	6.00 a		1.9597	14.00 a		2.9770
<i>B.t.</i>	8.25 b	26.19		22.00 b	28.26		2.67	20.00		1.6950	0.2760	4.33 b	27.78		8.33 b	40.48	
<i>T. evanescens</i>	7.40 b	33.86		20.67 b	32.61		2.00	40.00		1.7600	3.8600	3.67 b	38.90		6.67 b	52.38	
<i>T. evanescens</i> + <i>B.t.</i>	4.84 c	56.68		13.00 c	57.61		1.33	60.00		1.7733	4.5940	1.67 c	72.22		3.67 c	73.81	
In 2003																	
Control	10.84 a		1.2076	27.67 a		4.8615	3.00		---	1.8133	---	5.67 a		1.7188	16.67 a		2.4908
<i>B.t.</i>	7.70 b	28.19		17.33 b	37.35		2.33	22.22		1.8333	1.1030	3.67 b	35.00		9.67 b	42.00	
<i>T. evanescens</i>	6.67 b	38.45		15.00 b	45.78		1.67	44.44		1.8400	1.4720	3.33 b	41.00		7.33 b	56.00	
<i>T. evanescens</i> + <i>B.t.</i>	4.58 c	57.71		10.00 c	63.86		1.00	66.67		1.8600	2.5750	1.33 c	76.00		4.00 c	76.00	



The results proved that *Trichogramma* is a reliable and effective pest control agent for *O. nubilalis* in maize fields. *B.t.* treatments showed effective integration with *Trichogramma* without negative interaction between them. Inundate maize fields with the egg parasitoid, in proper time and in integration with *B.t.* can be diminished *O. nubilalis* attack to more than 70%, as mentioned by Bigler and Brunetti (1992). Mertz *et al.*, (1995) who found that *O. nubilalis* egg masses survival decreased by at least 45% in *Trichogramma brassicae* Benzdenko released plots. Other control options, however, are limited by the extreme sensitivity of adult *Trichogramma* to pesticide drift; e.g. *B.t.* is one of the options that had no toxicity to adult *Trichogramma* (Losey *et al.*, 1995).

From the above discussions, it could be concluded that the uses of both *T. evanescens* and *B.t.*, as two biological control agents reduced the number of European corn borer larvae per corn plant and its amount of damage significantly. This reduction was lower when either treatment was used alone. *T. evanescens* was not competitive with *B.t.* as a control agent for controlling *O. nubilalis* but they well integrated.

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استخدام طفيل *Trichogramma evanescens* والبكتيريا *Bacillus thuringiensis*

في مكافحة ثاقبة الذرة الأوروبية *Ostrinia nubilalis* في حقول الذرة الشامية

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تم عمل دراسات معملية وحقلية لتقويم كفاءة بعض عناصر مكافحة الحيوية على ثاقبة الذرة الأوروبية، *Ostrinia nubilalis* Hb. أحد الآفات الرئيسية التي تصيب نباتات الذرة الشامية في مصر، وذلك بإطلاق طفيل التريكوجراما *Trichogramma evanescens* (West.) بمفرده أو مع البكتيريا *Bacillus thuringiensis*، في محافظة كفر الشيخ لمدة ثلاث مواسم ٢٠٠٢، ٢٠٠١، و ٢٠٠٣. إستهدف إستخدام البكتيريا مكافحة يرقات الآفة التي لم يتم التطفل عليها في طور البيضة بالتريكوجراما. تم إختبار تأثير البكتيريا *B. thuringiensis* على الأطوار المختلفة للطفيل *T. evanescens* داخل بيض العائل تحت الظروف المعملية حيث ظهر خفض طفيف في نسبة خروج الطفيل البالغ بينما لم تتأثر إناث الطفيل الناتجة بعد المعاملة خاصة في عملية وضع البيض. استناداً على نتائج المواسم الثلاثة، كان متوسط نسبة التطفل الطبيعي بطفيل التريكوجراما على بيض *O. nubilalis* ٣٦،٠٠٠، ٣٨،٣٧، و ٣٦،٠٨٢% خلال ٢٠٠١، ٢٠٠٢، و ٢٠٠٣، على التوالي. بعد إطلاق إجمالي ٩٦٠٠٠ طفيل للقدان على دفعتين، بلغت نسبة التطفل ٧٤،٧٢، ٧٦،٨٣ و ٧٧،٢٣% في القطع التجريبية المعاملة بالطفيل بمفرده، بينما كانت ٧٤،٢١، ٧٢،٩٠ و ٧٥،٥٦% في القطع التجريبية التي تم إطلاق الطفيل فيها بالإضافة للبكتيريا، على التوالي. بلغت نسبة الخفض في أعداد يرقات *O. nubilalis* ١،٧٩ و ٧٣،٢١% في موسم ٢٠٠١ مقارنة بموسم ٢٠٠٢ حيث بلغت ٥٢،٣٨ و ٧٣،٨١% بينما في موسم ٢٠٠٣ فقد كانت ٥٦،٠٠ و ٧٦،٠٠% في القطع التجريبية التي تم إطلاق الطفيل فيها بمفرده و تلك المعاملة بالطفيل مع البكتيريا، على التوالي. تأثرت النسبة المئوية لعدد المعقل ومتوسط عدد القيوب والسنابل والكيوزان المصابة معنوياً في القطع التي تم إطلاق الطفيل فيها مع البكتيريا مقارنة بأعدادها في المقارنة.