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EVALUATION THE EFFECTIVENESS OF *Trichogramma evanescens* (WESTWOOD), *Bacillus thuringiensis* (BERL.) AND *Beauveria Bassiana* (BALS.) AS BIOLOGICAL CONTROL AGENTS FOR *Ostrinia nubilalis* (HUBNER) IN MAIZE FIELDS AT KAFR EL-SHEIKH DISTRICT.

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ABSTRACT

The effectiveness of *Trichogramma evanescens*, *Bacillus thuringiensis* and *Beauveria bassiana* against *Ostrinia nubilalis* on Nili maize plantations (during a period extended from July 15th to Nov. 15th) in 2001 and 2002 at Kafr El-Sheikh district was tested by releasing the egg-parasitoid, *T. evanescens* (two releases at a rate of 33000 parasitoids (release/feddan at 7-day interval) applying with *B. thuringiensis var. Kurstaki* (dipel 2x at a rate of 2.5 gm/L) and the fungus, *B. bassiana* at a rate of 5×10^7 conidia/ml. The mean percentages of infestation by *O. nubilalis* larvae at harvest were 12.25% and 9.3% showing reduction in infestation by 82.8 and 85.8%, respectively when *T. evanescens* was used in two seasons 2001 and 2002, two sprayings of *B. thuringiensis* treatment led to 20.34 and 15.22% showing reduction in infestation by 61.3 and 59.2%, whereas the mean percentages by the fungus were 29.21 and 23.32% showing reduction in infestation by 32.72 and 34.03, respectively as compared with control in 2001 and 2002.

Key words: *Trichogramma evanescens, Bacillus thuringiensis, Beauveria bassiana, Ostrinia nubilalis.*

INTRODUCTION

The European corn borer, *Ostrinia nubilalis* (Hubner) is an important economic insect pest to corn. In Egypt, Hosny and El-Saadany (1973) indicated that the economic damage of *O. nubilalis* infestation in maize plants ranged between 9 and 70% depending on sowing date.

Sherif and Lutfallah (1992) in Egypt reported 33.6% reduction in yield of maize plants grown on July, 25th that were subjected to relatively high infestation levels with *O. nubilalis*.

Metwally (2000) recorded two peaks of abundance for *O. nubilalis* which indicated two annual generations; the first occurred during June and July and it represented a minor one and slightly infested April and May plantations. The second one was noticed from August to October and represented the major one and heavily attacked July plantations at Kafr El-Sheikh.

As an alternative to chemical control or as part of IPM programs the use of *Trichogramma spp.* are active egg parasitoids of a wide range of lepidopterous pests and hence received considerable attention from numerous authors, Lingren 1970 and Parker *et al.*, 1971.

Hassan (1990) stated that, the efficacy of *Trichogramma spp.* against *O.nubilalis* eggs in the fields depends on parasitoid searching ability, host perference and tolerance to environmental conditions. *Bucillus thuringiensis* (Berl.) has an advantages over chemical insecticides as it integrates well with the use of other pest control methods (Barbosa, 1993).

Bartels and Hutchison (1995) used aerial applications of *B. thuringiensis sub. sp. Kurstaki* and tank-mixes with synthetic insecticides for control of *O. nubilalis* in Minnesota and they found that, treatments with low rate of Permethrin mixed with *B. thuringiensis* provided control of *O. nubilalis* equal to full rates of Permethrin.

The entomopathogenic fungi have long been known to cause epizootics among certain insects under both laboratory and field conditions (Barson *et al.*, 1994; Watson *et al.*, 1996 and Reithinger *et al.*, 1997). Many studies were carried out dealing with natural incidence laboratory and field trials for controlling eggs and larvae of *O. nubilalis* by *Beauveria bassiana* (Martel *et al.*, 1980; Hudon *et al.*, 1982; Hardin, 1991; Lewis and Bing 1991; Bing and Lewis 1991; Losey *et al.*, 1992; Bruck and Lewis 1999 and Wagner and Lewis 2000).

Therefore, the present investigation was carried out at Sakha Agricultural Research Station to test the efficacy of biocontrol agents including; *T. evanescens, B. thuringiensis* and *B. bassiana against O. nubilalis* in maize fields during two successive seasons 2001 and 2002.

MATERIALS AND METHODS

Field experiments were conducted in 2001 and 2002 Nili Plantation (extended from July 15th to Nov. 15th) to study the efficacy of *T. evanescens, B. thuringiensis* and *B. bassiana, for controlling O. nubilalis* in maize fields. Two sites of about ¼ feddan each at Sakha Agric. Research Station were chosen at a distance of 300 m between them to prevent migration of parasitoids from site of relase to other site. The area was divided into 16 equal plots that received 3 treatments and control of 4 replicates in a randomized block design. Giza 2 maize variety was sown at a rate of 2 seeds/hill on July, 15th. All plots received the recommended agricultural practices. Four plots of the site were left as a control whereas the other 4 plots received two releases of *T. evanescens*, 4 plots received two applications of Dipel 2x, the other 4 plots received two applications of the fungus *B. bassiana*. Egg-masses of *O. nubilalis* were counted weekly in 20 maize plants chosen randomly from late July till the end of the season, during 2001 and 2002.

The eggs of *Anagasta kuchniella* parasitized by *T. evanescens* were supplied from the laboratory of *Trichogramma* mass production, Biological Control Dept., Agric. Research Center (Abbas *et al.*, 1989). Release of *T. evanescens* took place on September 5th and 12th 2001 and on Sept. 2nd and 9th 2002. Each plot (128 m^2) received two releases at a rate of one strip (1000 parasitized eggs/release), indicating a rate of approximately 66.000 wasps/feddan. Each strip was hanged to a plant to avoid the destruction of the parasitized eggs by insect predators, the edge of carton was fixed in a way, which helps escaping the emerging parasitoids but prevents entrance of predators.

Bacillus thuringiensis variety Kurstaki at the recommended rate 2.5 g/litre and *Beauveria bassiana* at a rate of 5×10^7 condia/ml were used. Application of these agents were always carried out before sunset to avoid the adverse effect of the ultra-violet light during the day. Knapsack sprayer

model CP3, 20 litters was used for spraying by pushing the sprayer to stems of plants to give chance for the solution insecticides to go through the axils of the leaves where the newly hatched larvae were found. Examining of the plants took place after 7 days of applications and at two weeks intervals until harvest. Twenty plants were randomly cut from each plot and examined for the percentage of infested internodes, number of holes/100 internods and number of larvae/100 internodes.

Percent of reduction were estimated according to Henderson and Telton (1955).

Means for all data were compared by using L.S.D. method according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Data obtained in Table (1) indicated that, egg-masses of *O. nubilalis* began to appear on maize plants from mid of August until the fourth week of October in season 2001. A peak of 143 egg-masses/20 plant was recorded on September 12th. The infestation started to appear on August 22nd represented by 11 egg-masses/20 plants and reached a maximum of 117 egg-masses/20 plants on September 5th 2002 and gradually decrease until the third week of October.

Metwally (1979) reported that, this borer was very active and caused damage to maize sown from early June to the end of August at Kafr El-Sheikh. It is noted that, this pest attacks maize plants cultivated during July causing damage to both stalks and ears. The obtained results are in agreement with Isa and Awadallah (1975) and Tantawi (1981) they indicated that, the economic damage of *O. nubilalis* infestation in maize plants ranged between 9 and 70% depending on sowing date.

Data obtained in Table (2) showed that, the percentages of infested internodes, holes and numbers of larvae reached 17.3, 24.5, 31.1% when *B. t* (2.5g/L) was used. In case of *B. bassiana* these percentages were 26.3, 31.9 and 38.7%. Whereas using *T. evanescens* gave 15.2, 21.7 and 13.1%, respectively. The aforementioned data compared with the untreated maize as these percentages were 51.2, 64.17 and 73.45%, respectively.

Table (1): Weekly counts of *O. nubilalis* egg-masses/20plants during maize plantation of July 15, 2001 and 2002 at Kafr El-Sheikh district.

Date of inspection		2001	2002
July	30th	0	0
Aug	7th	0	0
	14th	5	0
	22nd	15	11
	29th	21	18
Sep.	5th	102	117
	12th	143	108
	19th	82	90
	26th	35	46
Oct.	2nd	23	26
	9th	11	9
	16th	8	14
	23rd	3	0
	30th	0	0

Table (2): Percentages of infested internodes, holes and larvae as affected by, *B. thuringiensis*, *B. bassiana* and *T. evanescens* on maize plants infested by *O. nubilalis* at Kafr EL-Sheikh region during 2001 season.

Treatments	Dosage of application	Parameter	* Percentage after treatments				
			20/9	5/10	20/10	5/11	Mean
B. thuringiensis	2.5 g/L	infestation	10.0	12.3	22.8	24.2	17.3
		holes	11.1	19.0	32.9	35.2	24.5
		larvae	22.8	29.2	35.2	37.5	31.1
B. bassiana	5×10^7 conidia/ml	infestation	13.8	20.5	34.3	36.8	26.3
		holes	13.2	25.3	35.9	53.5	31.9
		larvae	15.3	29.8	44.4	65.4	38.7
T. evanescens	66.000 wasps/feddan	infestation	9.9	18.4	15.4	17.2	15.2
		holes	9.5	23.6	25.4	28.4	21.7
		larvae	7.7	9.5	11.9	23.4	13.1
Check		infestation	17.5	45.7	65.5	76.2	51.2
		holes	22.0	55.2	79.3	100.2	64.17
		larvae	35.5	79.6	82.9	95.8	73.45

* Estimated as a number of infested internodes, holes and larvae per 100 internodes.

Data obtained in Table (3) indicated that, the percentages of infested internodes, holes and larvae were 18.5, 23.3 and 25.4% when *B.t* was used. Application of *B. bassiana* led to 29.2, 27.5 and 24.3%, but using *T. evanescens* gave 10.17, 14.3 and 7.5% as the percentages were 44.9, 53.8 and 53.2% in the untreated maize, respectively.

Table (3): Percentages of infested internodes, holes and larvae as affected by, *B. thuringiensis*, *B. bassiana* and *T. evanescens* on maize plants infested by *O. nubilalis* at Kafr EL-Sheikh region during 2002 season.

Treatments	Dosage of application	Parameter	* Percentage after treatments				
			23/9	9/10	22/10	7/11	Mean
B. thuringiensis	2.5 g/L	infestation	10.14	18.9	21.8	23.2	18.5
		holes	-	19.8	32.3	41.3	23.3
		larvae	15.6	18.3	35.5	32.3	25.4
B. bassiana	5×10^7 conidia/ml	infestation	22.5	33.7	31.2	29.5	29.2
		holes	15.6	25.4	29.7	39.6	27.5
		larvae	16.5	18.2	29.9	32.4	24.3
T. evanescens	66.000 wasps/feddan	infestation	7.4	8.7	11.2	13.4	10.17
		holes	6.2	10.2	18.4	22.4	14.3
		larvae	6.5	5.2	8.2	10.2	7.5
Check		infestation	19.9	42.7	47.8	79.2	44.9
		holes	24.2	42.9	60.2	88.2	53.8
		larvae	30.2	69.2	35.7	60.2	53.2

* Estimated as a number of infested internodes, holes and larvae per 100 internodes.

Data in table (4) indicated that the average of infested internodes, number of holes and number of larvae were significantly lower than that of untreated maize. The release of parasitoids was effective and superior in this respect. The percentage of reduction reached 82.9 in the infested internodes,

79.2 in holes and 80.2% in numbers of larvae. *B. thuringiensis* (at 2.5 g/L) arranged in the second order, as the percentage of reduction was 61.3 in case of infested internodes, 56.3 in holes and 56.2% in the recorded number of larvae. Treatment with *B. bassiana* at 5×10^7 conidia/ml came in the last order since percentage for internodes, holes and number of larvae reached 34.7, 28.2 and 42.6%, respectively.

Table (4): Effect of different applications of biocontrol agents, *B. thuringiensis, B. bassiana* and *T. evanescens* for controlling *O. nubilalis* at harvest 2001.

Treatments	Dosage of application	Infested internodes		Holes		Larvae	
		No.	Reduction	No.	Reduction	No.	Reduction
B. thuringiensis	2.5 g/L	20.34c	61.33	27.22c	56.34	28.91c	56.23
B. bassiana	5×10^7 conidia/ml	29.21b	34.72	43.62b	28.22	38.31b	42.67
T. evanescens	66.000 asps/feddan	12.25d	82.9	17.29d	79.2	12.13d	80.22
Check		48.22a	-	59.22a	-	67.72a	-

Averages followed by the same letters within a column did not differ significantly at 5% level.

Table (5) indicated that the results of season 2002 are very close to those of season 2001. *T. evanescens* was significantly more effective and recorded the percentage of infested internodes, number of holes and numbers of larvae by 85.8, 81.3 and 93.7%, respectively. In case of *B. thuringiensis* these percentages reached 59.2, 44.9 and 61.2%, respectively. When the fungus used these percentages reached 34.03, 35.9 and 46.5%, respectively.

Table (5): Effect of different applications of biocontrol agents, *B. thuringiensis, B. bassiana* and *T. evanescens* for controlling *O. nubilalis* at harvest 2002.

Treatments	Dosage of application	Infested internodes		Holes		Larvae	
		No.	Reduction	No.	Reduction	No.	Reduction
B. thuringiensis	2.5 g/L	15.22c	59.2	21.2b	44.92	18.2c	61.22
B. bassiana	5×10^7 conidia/ml	23.32b	34.03	25.9b	35.97	24.02b	46.55
T. evanescens	66.000 asps/feddan	9.26b	85.8	12.9c	81.3	6.8d	93.7
Check		32.32a	-	40.3a	-	43.62a	-

According to L.S.D. values of the two years, the tested treatments could be arranged according to their efficacy in a descending order as follows; *Trichogramma, B. thuringiensis, B. bassiana* and control. Such data indicated significant differences between the control and the other three treatments also, significant differences were detected between the three treatments. Regarding the percentage of reduction in *O. nubilalis* larval counts due to the different treatments, it is clear that all the treatments caused considerable reduction at harvest.

Lewis *et al.*, (1971) referred to the ways of developing *Trichogramma spp.* as a control agent to certain agricultural pests. They stated that the most

commonly suggested method to achieve this goal is to spray the crops with eggs of easy to rear nonpest insect, such as *Sitotroga cerealella*.

Neuffer (1980) reported that, the release of *Trichogramma evanescens* (west.) at rates of 50.000 to 100.000 wasps/hectare at the time of stem borer ovipositions lead to high reduction in infestation especially at the highest rates of release.

Dicky and Bayers (1994) indicated that at a releasing level of about 196.000 wasps of *T. brassicae* per ha for control of *O. nubilialis*, the reduction in damage ranged from 45 to 95%.

Currier and Gawron (1989) stated that *B. thuringiensis* Berl. and other bacterial insecticides must be ingested by a susceptible insect host to be effective.

Georghiou and Legunes (1991) stated that, *B. thuringiensis* is the ideal means of controlling lepidopterous pests in agriculture because of many attributes that differentiate this microbial insecticide from the synthetic chemical formulations.

Mc Gurire *et al.*, (1994) used two types of pregelatinized maize flour to produce granules containing *B. thuringiensis subsp. Kurstaki* and various additives for controlling *O. nubilalis* in the whorl of maize plants.

Many authors stated that, the entomopathogenic fungus, *Beauveria bassiana* was used to suppress the larval populations of *O. nubilalis* in maize fields (Bartlett & Lefebure 1934; Beall *et al.*, 1939; York 1958; Riba 1984; Marchandier & Riba 1986).

Bing and Lewis (1991) found that *B. bassiana* on maize plants provided 98.3% reduction in *O. nubilalis* infestation.

In conclusion, using *T. evanescens* (West.) is an important excellent biocontrol agent. It attacks the host while the later is immobile. Besides, *B. thuringiensis* (Berl.) and *B. bassiana* (Bals.) as microbial insecticides are much effective and safe. It is possible to minimize the larval content of the target pest and to share in making better environment free from insecticidal hazards. Consequently, it can be recommended for applying against *O. nubilalis* at the whorl stage of maize plants, 40 days after sowing, to be coincide with the highest population of borer egg-masses especially for Nily (July) plantation.

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تقييم فاعلية طفيل التريكوجراما وبعض المبيدات الميكروبية (البكتريا الثورنجينية، وفطر البيوفاريا باثيانا) كعوامل مكافحة حيوية ضد ثاقبة الذرة الأوربية فى الحقل خلال موسمى ٢٠٠١ و٢٠٠٢م فى منطقة كفرالشيخ.

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قسم بحوث المكافحة الحيوية - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - مصر

تم تقييم فاعلية طفيل التريكوجراما وبعض المبيدات الميكروبية (البكتريا الثورنجينية، وفطر البيوفاريا باثيانا) ضد ثاقبة الذرة الأوربية على زراعات الذرة فى الفترة من ١٥ يوليو الى ١٥ نوفمبر خلال موسمى ٢٠٠١ و ٢٠٠٢ فى منطقة كفرالشيخ وقد أظهرت النتائج أن إطلاق الطفيل بمعدل ٣٣٠٠٠ طفيل للفدان مرتين كل اسبوع على أن يكون بين المدة والأخرى اسبوع أدى الى خفض الإصابة الى ٨٢,٨، ٨٥,٨% وعند استخدام المبيد البكتيرى انخفضت الإصابة بنسبة ٦١,٣ و ٥٩,٢% أما فى حالة استخدام المبيد الفطرى فوصلت نسبة الخفض الى ٣٢,٧ و ٣٤,٠% وذلك مقارنة بالكنترول فى عامى ٢٠٠١ و ٢٠٠٢ على الترتيب.