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Efficacy of a bacterial insecticide, on unparasitized and parasitized lesser cotton leafworm *Spodoptera exigua* Hbn. larvae by the endoparasitoid *Microplitis rufiventris* Kok.

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Abstract

Laboratory studies were conducted to evaluate the toxic effect of the bioinsecticide Biotecht against the beginning of the third instar larvae of the lesser cotton leafworm *Spodoptera exigua* Hbn. (either unparasitized or parasitized ones by the endoparasitoid *Microplitis rufiventris* Kok.). The LC_{50} values obtained for both larvae were; 13.703×10^4 and 17.414×10^4 I.U., respectively (computed from 72 hours mortality data).The mortality percentages were higher in case of unparasitized larvae comparing with the parasitized ones (where, the parasitized larvae were less susceptible to the bioinsecticide treatment than the unparasitized ones).This result was also emphasized by the total daily amounts of food consumed by both pest larvae. Therefore, using the bioinsecticide Biotecht may be recommended and could be combined with releasing the parasitoid *M. rufiventris*. They could be used side by side with other available safe control methods, when planning for Integrated Pest Management (IPM) strategies against the lesser cotton leafworm *S. exigua*.

Key Words: Bacterial insecticide, *Bacillus thuringiensis kurstaki*, *Spodoptera exigua*, Bioassay, Parasitism, *Microplitis rufiventris*

Introduction

In Egypt, the lesser cotton leafworm *Spodoptera exigua* Hbn. (Lepidoptera: Noctuidae) is a serious pest allover the year round. The larval instars are polyphagus, their feeding cause economic damage on more than 50 plant species from over 10 families such as corn, cotton, soybean, lettuce, alfalfa and tomato (Greenberg *et al.,* 2001).

However, natural enemies such as parasitoids play an important role in suppressing their population density (El-Khawas and Shoeb, 2006).The solitary larval endoparasitoid *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae), is one of these parasitoids that parasitize many lepidopterous pests' including the lesser cotton leafworm *S. exigua* (El-Minshaway, 1963 and El-Moursy *et al.*, 2000).

The indiscriminate and extensive use of chemical insecticides had lead to environmental pollution and toxicity of mammals and beneficial organisms (Ahmed, 1998; Salwa *et al.* 2001 and Abdel-Rahman *et al.*, 2005). Therefore, the recent Integrated Pest Management (IPM) strategies developed for insect control are mainly concentrated on the use of all suitable safe control methods, to maintain pest's population under the economic injury level and also, to reduce the environmental pollution. Biological control has received much crucial worldwide and revealed significant impact as possibly safe mean of insect control (Kares, 1991; Rashed, 1993; and Sabbour &Abbas, 2007).

Recently, microbial insecticides as a component of biological control technique are developed and encouraged. Their uses give good results against pests without polluting the environment. Besides, their toxic effect is very low on non-target animals and humans (Aranda *et al.*, 1996). The most abundant and successful microorganism used as effective bioinsecticide was *Bacillus thuringiensis* Berliner (Carlton, 1988; De Maagd *et al.,* 2001 and Ibrahim & Omar, 2005).The tolerance effect of *B. thuringiensis* comes from the δ-endotoxin formed during sporulation , which is also toxic to insect larvae of order Lepidoptera .

The present study was conducted under laboratory conditions and aims to evaluate the toxic effect of a commercial microbial insecticide (Biotecht, containing *B. thuringiensis kurstaki*), on unparasitized and parasitized *S. exigua* larvae by the endoparasitoid *M. rufiventris*. This information is needed to be considered, when both the bioinsecticide and the parasitoid are applied as biological control components in IPM programs against the lesser cotton leafworm *S. exigua.*

Material and methods

The lesser cotton leafworm *S. exigua* and its parasitoid *M. rufiventris* (cocoons and adults), were collected from cotton fields and vegetable crops in Qalubia Governorate. Pest larvae were successfully reared under the laboratory conditions of 27±2 °C and 65±5 % R.H., on castor-bean leaves (*Ricinus communis* (L.))

I-A- Rearing of the parasitoid *M. rufiventris*:

The parasitoid *M. rufiventris* was reared, according to the technique previously described by Kares *et al.* (1998), on the cotton leaf worm *S. littoralis* as a host.

B- Individual parasitism of the lesser cotton leafworm *S. exigua* larvae by the parasitoid *M. rufiventris*:

Also, the parasitism by the parasitoid *M. rufiventris* on the lesser cotton leafworm *S. eixgua* followed the technique described by Kares *et al.* (1998), on *S. littoralis* as a host

II- Bioinsecticide used:

The bioinsecticide Biotecht 9.4%W.P. is a selective bacterial insecticide containing 32×10^6 I.U. of *B. thuringiensis* var. *kurstaki*/gm. of product. It is a product developed by Organic Biological Technology Company. Also, it was recommended to be applied against the newly hatched larvae of the cotton leaf worm *S. littoralis*, at a rate of 300 grams/feddan.

III- Treatments:

Weights of 0.063,0.125,0.250,0.500,1.00 and 2.00 grams of Biotecht were diluted in distilled water to obtain a constant volume of 100 ml.(total volume), to represent the dilutions of 2×10^4, 4×10^4, 8×10^4, 16×10^4, 32×10^4 and 64×10^4 I.U., respectively.

The following procedures were followed:

1-Three replicates, each of ten newly molted third instar larvae of the lesser cotton leafworm *S. exigua* either unparasitized or parasitized ones by the parasitoid *M. rufiventris* were tested.

2-Fresh castor–bean leaves were dipped for one minute in the different dilutions of the bioinsecticide, then, these treated leaves were left for an hour for air dryness.

3- The ten newly molted third instar larvae of the lesser cotton leafworm *S. eixgua* either unparasitized or parasitized, were kept in plastic cups (7.5×4 cm.),with perforated plastic covers. They were allowed to feed for 48 hours on the treated castor-bean leaves (that previously dipped in the six different dilutions of the bioinsecticide). Surviving larvae (either unparasitized or parasitized ones), were transferred to other clean plastic cups containing untreated castor-bean leaves until either pupation for unparasitized larvae or formation of parasitoid cocoons in case of parasitized ones. The control test was conducted using the same source of food, but dipped only in water.

4-Before exposing the larvae to treated food, they were starved for 6 hours in order to obtain rapid ingestion of the contaminated food.

5- Experiments were carried out under the laboratory conditions of 27±2°C and 65±5 % R.H.

IV- Effect of parasitism on the amount of food consumed by *S. eixgua* parasitized by *M. rufiventris*:

This experiment was conducted according to the technique described by Kares *et al.*(1998), using *S. littoralis* as a host. It was made to explain the obtained data in this study, concerning the difference between mortality percentages of unparasitized *S. exigua* larvae and parasitized ones by the parasitoid *M. rufiventris*.

V-Statistical analysis: The effectiveness of different treatments was expressed in terms of LC_{50} values at 95 fiducially limit slopes of regression lines. Statistical analysis of the obtained data were made based on the analysis of variance and liner regression analysis (Finney, 1971 and slide write program)

Results and discussion

I- Effect of the bioinsecticide on unparasitized and parasitized *S. exigua* larvae:

Daily mortalities among treated third instar unparasitized *S. eixgua* larvae are shown in Table(1) and illustrated in Fig.(1). The mortality percentages after 72 hours of treatment (at which LC_{50} was estimated) ranged between 16.67 and 80.00 %, at dilutions between 2×10^4 and 64×10^4 I.U., respectively. The LC_{50} value was 13.703×10^4 I.U. (Table, 2). Also, obtained data revealed that, the mortality percentages increased as a result of increasing the concentrations of the tested bioinsecticide. Results agreed with findings of Kares *et al.* (1992) and El-Khawas (2000 & 2001).

In addition, results obtained from third instar parasitized larvae by the parasitoid *M. rufiventris* that were fed on castor-bean leaves treated with the six different dilutions of the bioinsecticide, were also summarized in Table (1) and illustrated in Fig. (1).The corrected mortality percentages after 72 hours (at which LC_{50} was estimated) ranged between 10.00 and 63.33 %, at dilutions between 2×10^4 and 64×10^4 I.U., respectively. The LC_{50} value was 17.414×10^4 I.U. (Table, 2). Also, obtained data revealed that, the mortality percentages after treatment were positively correlated with increasing the concentrations of the tested bioinsecticide.

Moreover, data shown in Table (1) indicated that, the mortality percentages of both unparasitized and parasitized S. *exigua* larvae increased gradually by increasing the time after bioinsecticidal treatment. In general, from Tables (1&2) and Fig. (1), it could be

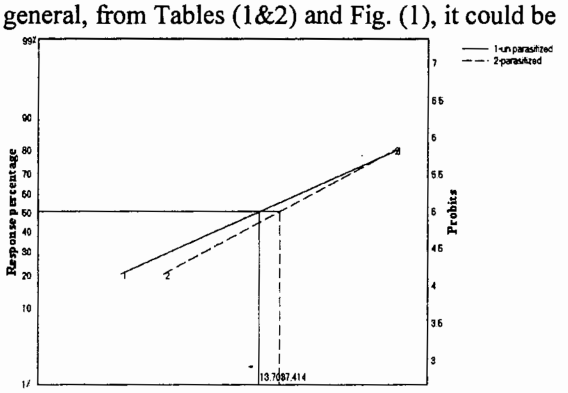

FIG (1): Log concentration Propit-line showing response of unparasitized and parasitized third instar larvae of S. *exigua* by the parasitoid *M. rufiventris*, to different dilutions of Biotecht (Computed from mortality data, 72 hours after treatment).

Table 1. Corrected mortality percentages of unparasitized and parasitized *S. exigua* larvae by the parasitoid *M. rufiventris*, fed on castor-bean leaves treated with a bacterial insecticide (Biotecht).

Dilutions (I.U.)	% cumulative mortality after days of treatment								
	1	2	3	4	5	6	7	8	9
				Unparasitized					
0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33	3.33	All surviving larvae reached the pupal stage
2×10^4	3.33	6.67	16.67	20.00	30.00	33.33	36.67	40.00	
4×10^4	6.67	13.33	26.67	33.33	40.00	46.67	53.33	60.00	
8×10^4	13.33	26.67	40.00	46.67	53.33	60.00	63.33	66.67	
16×10^4	20.00	33.33	50.00	60.00	66.67	70.00	73.33	80.00	
32×10^4	33.33	46.67	66.67	70.00	73.33	76.67	80.00	93.33	
64×10^4	46.67	50.00	80.00	83.33	86.67	93.33	100.0	100.0	
				Parasitized					
0.00	0.00	0.00	0.00	0.00	3.33	3.33	3.33	Forming of full grown larval parasitoids and pupate inside their cocoons	All host larvae died
2×10^4	0.00	3.33	10.00	16.67	26.67	30.00	33.33		
4×10^4	3.33	10.00	20.00	30.00	36.67	43.33	43.33		
8×10^4	10.00	16.67	30.00	36.67	40.00	46.67	50.00		
16×10^4	16.67	20.00	43.33	50.00	56.67	63.33	63.33		
32×10^4	26.67	33.33	56.67	60.00	63.33	66.67	70.00		
64×10^4	36.67	46.67	63.33	70.00	73.33	76.67	83.33		

Table 2. Comparative toxicity to third instar larvae of unparasitized and parasitized S. *exigua* by the parasitoid *M. rufiventris*, fed on castor-bean leaves treated with a bacterial insecticide (Biotecht).

S. exigua larvae	LC_{50}	Slope	Confidence limits at P0.05 of LC_{50}
Unparasitized	13.703×10^4	1.179 ± 0.112	$11.113 \times 10^4 : 17.073 \times 10^4$
Parasitized	17.414×10^4	1.411 ± 0.119	$14.522 \times 10^4 : 21.218 \times 10^4$

*Computed from mortality data, 72 hours after treatment.

concluded that the parasitized S. *exigua* larvae by the parasitoid *M. rufiventris* were less susceptible to the bacterial insecticide treatment than the unparasitized ones of the same age. Where, the corrected mortality percentages were higher in case of unparasitized *S. exigua* larvae comparing with parasitized ones. These results agreed with those of Shalaby *et al.* (1986); El-Moursy *et al.* (2000) and Kares (1991).

II- Effect of parasitism by *M. rufiventris* on the amount of food consumed by parasitized *S. exigua*:

The corrected mortality percentages were recorded to be higher in case of unparasitized *S. exigua* larvae comparing with the parasitized ones by the parasitoid *M. rufiventris* (Table, 1).

An experiment was made to estimate the effect of parasitism on the amount of food eaten by unparasitized and parasitized *S. exigua* (during the period of parasitoid existence in the host larvae).The obtained data (Table, 3) indicated that, the total amount of food consumed by the unparasitized *S. exigua* larvae were higher, comparing with those of parasitized ones (the general ratio was 3.45:1).This result was in coincidence with the percentages of corrected mortality recorded in Table (1) as follows:

1. Slight difference in the amount of food consumed among the unparasitized *S. exigua* larvae), comparing with parasitized ones, existed throughout the first 48 hours of treatments. This could be attributed to the fact that the parasitoid was still in the egg stage and that both the unparasitized and parasitized larvae consumed approximately the same quantity of castor-bean leaves at the same age.
2. From the third to the seventh day of parasitism, the amount of food consumed in case of the unparasitized *S. exigua* larvae were higher, compared with that of parasitized ones by the parasitoid *M. rufiventris*. This amount of food consumed showed either high or very high level differences between the unparasitized and parasitized larvae.

3. The two days after emergence of the full grown larval parasitoid (eighth and ninth days), the amount of food consumed in case of the unparasitized *S. exigua* larvae was high and different significant between the unparasitized *S. exigua* larvae and parasitized ones. However, the parasitized pest larvae ceased feeding on the eight,h day after emergence of full grown larval parasitoids till the host death.

Table 3. Effect of parasitism by the parasitoid *M. rufiventris* on the amount of food consumed by *S. exigua* larvae.

Days after parasitization	Mean weight of eaten castor-bean leaves(gram/day)/larva		Ratio of unparasitized to parasitized larvae
	Unparasitized	Parasitized	
1	0.020	0.022	1.00:1
2	0.050	0.047	1.06:1
3	0.091	0.060	1.52:1
4	0.119	0.075	1.59:1
5	0.171	0.080	2.14:1
6	0.182	0.063	2.89:1
7	0.200	0.021	9.52:1
8	0.211	0.000	0.21:0
9	0.218	0.000	0.22:0
Total	1.262 (0.020-0.218)	0.368 (0.000-0.080)	General ratio 3.43:1

These results are compatible with previous results shown in Table (1) that:-

1. The mortality percentages of unparasitized and parasitiszed larvae are nearly equal during the first 48 hours of treatment in all dilutions.
2. From the third to seventh day after larval feeding on contaminated food, the mortality percentages of parasitized *S. exigua* larvae was less than unparasitized ones in all dilutions.
3. In the eighth and ninth days after the emergence of the full grown larval parasitoid, the parasitized *S. exigua* larvae died. The survived unparasitized ones reached the pupal stage.

Conclusion

It could be concluded from the present study that, the bioinsecticide Biotecht could be recommended to be used at low dilutions as a stomach poison against the lesser cotton leafworm *S. exigua*. Besides, having the possibility to release at certain times the parasitoid *M. rufiventris* against this insect pest. The application of the bioinsecticide and the release of the parasitoid could be taken into consideration when planning future Integrated Pest Management (IPM) strategies against the pest. Those could be used side by side with other safe control methods, thus, helping to decrease the pollution of the surrounding environment.

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تأثير المبيد البكتيرى (Biotecht) ، على اليرقات الغير متطفل عليها و المتطفل عليها لدودة ورق القطن الصغرى *Spodoptera exigua* Hbn. بالطفيل الداخلى *Microplitis rufiventris* Kok.

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أجريت دراسة معملية لتقدير تأثير المبيد الحيوى Biotecht (مبيد بكتيرى ، البكتيريا *Bacillus thuringiensis* var. *kurstaki*) ،على دودة ورق القطن الصغرى .Spodoptera exigua Hbn فى بداية عمرها اليرقى الثالث الغيرمتطفل عليه و المتطفل عليه بالطفيل الداخلى *Microplitis rufiventris* Kok.

أوضحت النتائج أن نسب الموت كانت أعلى فى حالة اليرقات الغير متطفل عليها مقارنة بالأخرى المتطفل عليها ، وذلك عند تغذية اليرقات على غذاء معامل بالمبيد البكتيرى. وقد تم تقدير التركيزات المميتة ل٥٠% من يرقات الآفة ، حيث بلغت ١٣,٧٠٣X١٠ ⁴ و ١٧,٤١٤X١٠ ⁴ وحدة دولية ، على التوالى.

تم حساب كمية الغذاء اليومية التى تناولتها اليرقات غير متطفل عليها و المتطفل عليها بالطفيل (دون المعاملة بالمبيد). فوجد أنه قد تساوت كمية الأكل خلال ٤٨ ساعة الأولى بعد المعاملة لكلا" من نوعى اليرقات. بينما إبتداءا" من اليوم الثالث حتى نهاية التجربة (إما خروج الحشرات الكاملة للطفيل فى حالة اليرقات المتطفل عليها أوتكوين العذارى لتلك اليرقات الغير متطفل عليها) ، فقد أظهرت النتائج أن اليرقات الغير متطفل عليها تناولت كمية غذاء أعلى مقارنة باليرقات الأخرى المتطفل عليها. حيث بلغت نسبة الأكل الكلية بين اليرقات الغير متطفل عليها و اليرقات المتطفل عليها ٣,٤٣ : ١.

ولذا فإنه يمكن الإستفادة مما سبق ، بإطلاق الطفيل *Microplitis rufiventris* أولا" ثم بعد ذلك يتبعه إستخدام المبيد البكتيرى (Biotecht) ، لمكافحة دودة ورق القطن الصغرى على المحاصيل الزراعية المختلفة التى تهاجمها الآفة. ويوصى بإستخدامهما مع الوسائل الأخرى المتاحة والأمنة عند عمل برامج مكافحة متكاملة للآفة.