## Microbiology

# EFFECT OF BACILLUS THURINGIENSIS ON APANTELES RUFICRUS PARASITIZING THE LARVAE OF AGROTIS YPSILON

## M. HAFEZ\* H. S. SALAMA\*\* R. ABOUL-ELA\* F. N. ZAKI\*\* M. RAGAEI\*\*

SUMMARY: The interaction between the insect pest, Agrotis ypsilon; the parasite, Apanteles ruficrus and the pathogen Bacillus thuringiensis has been investigated. A. ruficrus parasitizing on host larvae treated with B. thuringiensis showed significant reduction in the egg production, formed cocoons, longevity of either sex and emerged adults. The mortalities caused by B-exotoxin were increased among parasitized host larvae more than the unparasitized ones.

Key Words: Bacillus thuringiensis, agrotis ypsilon.

## INTRODUCTION

The relation between the bacterial pathogen, Bacillus thuringiensis and the parasites of some insect pests has been investigated by several authors (3,5,6). They studied the deleterious effects caused by the pathogen to the parasites. In Egypt, a serious of investigations has been carried out to explore the possible interrelation between, the pathogen B. thuringiensis and the parasites of some insect pests (7-9,12-14).

In the present work, investigations have been carried out to determine the effect of the pathogen B. thuringiensis S-endotoxin on the larval parasite Apanteles ruficrus, associated with the greasy cutworm Agrotis ypsilon.

In addition, the effect of B-Exotoxin (thuringiensis) on the parasite has also been demonstrated.

## MATERIALS AND METHODS

Effect of B. thuringiensis on A. ruficrus : The effect of B. thuringiensis on the parasite was assessed by two different methods. In the first method, 100 newly moulted 3rd instar larvae of A. ypsilon were fed on artificial diet (1) containing B. thuringiensis at 500 µg/ml concentration. The larvae were then exposed to mated females of the parasite. In the second method, 100 host larvae were fed on fresh artificial diet (1) and then exposed to mated females of the parasite fed 10% honey solution containing B. thuringiensis Kurstaki, HD1 at the rate of 500 µg/ml. In the control, the host larvae were raised on untreated diet and exposed to the untreated parasites fed on 10% honey solution. In all cases, the exposure of larvae lasted for 24 hours at the rate of one couple of the parasite for each 10 host larvae.

Effect of B-exotoxin (thuringiensin) on A. ruficrus : Different concentrations of thuringiensin (provided by Abott Laboratories) (63, 125, 250 and 500 ppm) were prepared. The host diet was prepared according to Abdel-Salam, with the omission of auromycin. For each concentration 50 larvae were used. Larvae were separated and maintained at 26°C and 55% R. H. Mortality records were made after 7 and 14 days. The emerged adults of the parasite were isolated for further observations.

<sup>\*</sup> From Department of Entomology, Faculty of Science, Cairo University, Cairo, Egypt.

<sup>\*\*</sup> From Plant Protection Department, National Research Center, Dokki, Cairo, Egypt.

	Untreated parasites	Parasites fed on		
ýltem		Host larvae treated with B.t.	Diet with B. thuringiensis	
No of parasitized host larvae	$5.8\pm0.38$	$5.6\pm0.36$	$5.20\pm0.81$	
Adult longevity in days, females	9.3 ± 0.62	9.1 ± 0.57	$8.80\pm0.57$	
Adult longevity in days, males	$6.8\pm0.42$	$6.8\pm0.40$	$6.00\pm0.38$	
Number of deposited eggs/female	$25.7\pm1.42$	24.9 ± 1.17	$23.1\pm1.08$	
Duration (egg-pupa) in days	$18.0\pm1.32$	$26.2\pm2.74$	$24.80 \pm 1.42$	
% formed cocoons/host larvae	33.3	12.7	19.6	
% of emerged adults	75.5	14.3	49.3	
Longevity of emerged adult females	$8.9 \pm 0.94$	$5.2\pm0.38$	$6.30\pm0.71$	
Longevity of emerged adult males	$6.0\pm0.38$	$3.3\pm0.30$	$4.30\pm0.72$	

Table 1: Effect of B. thuringiensis on the biology of the endoparasite A. ruficrus reared on larvae of A. ypsilon.

## RESULTS

Apanteles ruficrus (Hall.) is gregarious internal braconid parasite of many noctuid larvae including the greasy cutworm Agrotis ypsilon (2,4,17).

Effect of B. thuringiensis on A. ruficrus: The number of larvae of A. ypsilon that have been parasitized by A. ruficrus was not affected whether the adult parasite was fed on honey solution containing B. thuringiensis or when the host larvae were fed on a diet treated with the pathogen. In the control, the average number of host larvae parasitized by a single Apanteles female was 5.8, compared to an average of 5.6 and 5.2 individuals after treating the host larvae and adult parasites by B. thuringiensis. The adult longevity showed insignificant changes as a result of treatment being 9.3 and 6.8 days for the normal females and males, compared to 8.8 and 6.0 days, respectively, after feeding females and males on honey with the pathogen. The longevity of adult parasites feeding on treated larvae also showed no obvious differences compared with the control (Table 1).

The egg production of A. ruficrus females was not affected as a result of both treatments when compared with the untreated individuals. Thus, the average egg production was 24.9 and 23.41 eggs/female for individuals parasitizing treated host larvae or adult parasites fed on B. thuringiensis, respectively, compared with an average of 25.7 eggs for the control female. The duration of the immature stages of the parasite (egg-pupae) was significantly prolonged as a result of treatment of the host larvae or feeding the parasite females on a diet containing B. thuringiensis, being 26.2 and 24.8 days compared with 18.0 days in the control. The percentage of parasites that completed their development on treated A. ypsilon larvae showed to be 12.7%, while it was 19.6% in case of treatment of the adult parasite with the pathogen, this is compared with 33.3% in the control.

Table 2: Susceptibility of unparasitized and A. ruficrus parasitized A. ypsilon larvae to B-exotoxin (thuringiensin).

State of host larvae	No of days after treatment with B-exotoxin	LC <sub>50</sub> of B-exotoxin in ppm
Unparasitized host larvae + B-exotoxin	7	131.52
Parasitized host larvae + B-exotoxin	7	146.88
Unparasitized host larvae + B-exotoxin	14	61.20
Parasitized host larvae + B-exotoxin	14	64.86

Journal of Islamic Academy of Sciences 8:1, 33-36, 1995

#### BACILLUS THURINGIENSIS

B-exotoxin conc. (ppm)	% mean mortality			
	Unparasitized	Parasitized		
None	16	71		
63	27	92		
125	38	89		
250	73 95			
500	95	98		

Table 3: Effect of B-exotoxin (thuringiensin) on unparasitized and A. ruficrus parasitized A. ypsilon larvae.

The emergence of the adult parasites was affected as a result of treatment by B. thuringiensis, where 14.3 % emergence occurred from treated parasitized host larvae compared to 75.5 % from the resulting cocoons in the control. Feeding the parasite on honey containing B. thuringiensis inhibits adult emergence being 49.3 %. On the other hand, a marked decrease was observed in the longevity of the parasites obtained from treated host larvae being 5.2 and 3.3 days for females and males, respectively. The average longevity of parasites fed on a diet containing the pathogen were less affected being 6.71 and 4.3 days for females and males, respectively, compared with an average of 8.9 and 6.0 days in the control (Table 1).

Effect of B-endotoxin (thuringiensin): The dosage mortality response to B-exotoxin (thuringiensin) for the Agrotis ypsilon larvae, showed that the 7-day-LC<sub>50</sub> values for unparasitized and parasitized individuals

were 131.52 ppm and 146.88 ppm, while the 14-day-LC50 values for unparasitized and parasitized individuals were 61.20 and 64.86 ppm, respectively (Table 2).

Table 3 illustrates, A. ypsilon larval mortalities after various treatments over the entire period of the host larval development. The unparasitized (control) larvae incurred 16% mortality during the entire life span, compared to 71% in the parasitized group.

The data indicate that the combined effect of thuringiensin and parasitism, showed an obvious increase than if treated with thuringiensin alone, especially at concentrations of 63, 125 and 250 ppm, where 92, 89 and 95 compared to 27, 38 and 73, respectively.

The effect of thuringiensin on A. ruficrus is presented in Table 4. In untreated larvae, cocoons of the parasite were formed after 12.5 days compared to 12.9, 13.6, 13.9 and 16.8 days of parasitizedthuringiensin treated larvae at the concentrations of 63, 125, 250 and 500 ppm.

In the untreated A. ruficrus, adults emerged from cocoons after 16.5 days, compared to 18.1 and 19.5 days of cocoons of parasitized-thuringiensin treated larvae at concentrations of 250 and 500 ppm, respectively (Table 4).

The foregoing results revealed that, treatment of the host larvae A. ypsilon with B. thuringiensis did not change their susceptibility to parasitism by A. ruficrus. The longevity of the adult parasite and its egg production showed no obvious differences as a result of the

B-exotoxin conc. (ppm) host larvae	A. ruficrus cocoons		A. ruficrus adults		
	Total no.	Mean formation time (days)	Total no.	Mean emergence time (days)	
None	100	67	$12.5\pm1.17^{a}$	38	$16.5\pm1.26^{a}$
63	100	39	$12.9 \pm 1.28^{a}$	20	$16.8\pm1.62^{a}$
125	100	20	$13.6\pm1.42^{a}$	7	$17.3 \pm 1.34^{a}$
250	100	7	$13.9\pm0.73^{a}$	4	$18.1\pm1.60^{a}$
500	100	3	$16.8\pm1.13^{\text{b}}$	2	$19.5\pm1.27^{\text{b}}$

Table 4: Effect of B-exotoxin treatment of A. ypsilon host larvae on the development of the parasite A. ruficrus.

Means of each column followed by the same letter are not significantly different at 5% level.

Journal of Islamic Academy of Sciences 8:1, 33-36, 1995

#### BACILLUS THURINGIENSIS

#### HAFEZ, SALAMA, ABOUL-ELA, ZAKI, RAGAEI

treatment compared with the control. The duration of the immature stages of the parasite was significantly prolonged as a result of feeding the host larvae or the parasite on a diet with B. thuringiensis. The formation of cocoons and the emergence of the adults were markedly decreased.

Investigations showed that the exotoxin (thuringiensin) causes similar effects like B. thuringiensis on the parasite.

Results obtained on the interrelation between the pathogen and the parasites Microplitis demolitor, Zele chlorophthalma, Trichogramma evanescens and Bracon brevicornis showed significant reduction in the egg production, formed cocoons, emerged adults and their longevities (7,8,10,11,13). Previous histopathological studies on the larvae of Spodoptera littoralis showed that the pathogen caused changes in the fat bodies and some other organs which are necessary to the normal development of the parasite (7,8).

#### REFERENCES

1. Abdel-Salam NM : Bionomic studies on the greasy cutworm Agrotis ypsilon (Lepidoptera: Noctuidae). Ph.D. in Agricultural Sciences Fac Agric, Ain Shams Univ, pp 24-29, 1987.

2. Bishara I : The greasy cutworm Agrotis ypsilon Rott in Egypt. Bull No 114, Tech and Scient Service, Mon Agric Egypt, 1932.

3. Bracken GK and Bucher GE : Mortality of hymenopterous parasite caused by Serratia mercescens. J Invertebr Pathol, 9:120-132, 1967.

4. Hafez M : The biology and life history of Apanteles ruficrus. Bull Soc Found 1er Ent, 31:252-259, 1947.

5. Kaya H, Dunbar D, Doane C, Weseloh R and Anderson J : Gypsy moth: Aerial tests with Bacillus thuringiensis and pyrethroids. Conn Agric Exp Stn New Haven Bull, p 744, 1974.

6. Laigo FM and Tamashiro M : Interactions between a microsporidium pathogen of the lawn-armyworm and the hymenopterous parasite Apanteles marginiventris. W Invertebr Pathol, 9:546-554, 1967.

7. Salama HS and Zaki FN : Interaction between Bacillus thuringiensis Berl and the parasites and predators of Spodoptera littoralis in Egypt. Z Angew Entomol, 95:425-429, 1983.

8. Salama HS and Zaki FN : Histopathological effects of Bacillus thuringiensis on the larvae of Spodoptera littoralis (Boisd). Annals Agric Sci Fac, Agric Ain Shams Univ, 28:301-318, 1983. 9.Salama HS and Zaki FN : Impact of Bacillus thuringiensis on the predator complex of Spodoptera littoralis in cotton fields. Z Ang Ent, 87:485-490, 1984.

10. Salama HS and Zaki FN : Biological effects of Bacillus thuringiensis on the egg parasitoid, Trichogramma evanescens. Insect Sci Applic, 61:145-148, 1985.

11. Salama HS, Zaki FN and Sharaby AF : Effect of Bacillus thuringiensis Berl on parasites and predators of the cotton leaf worm Spodoptera littoralis. Z Ang Ent, 64:498-504, 1982.

12. Salama HS, Salem S, Zaki FN and Matter M : Control of Agrotis ypsilon (Lep: Noctuidae) on some vegetable crops in Egypt using the microbial agent Bacillus thuringiensis. Anz Shadl Unwelt, 36:147-151, 1990.

13. Salama HS, EL-Moursy A, Zaki FN and Abd El-Razek A : Parasites and predators of the meal moth Plodia interpunctella Hbn, as affected by Bacillus Thuringiensis. J Appl Entr, 112:244-253, 1991.

14. Salama HS, Zaki FN, Salem S and Ragaei M : The use of Bacillus thuringiensis to control two lepidopterous insect pests (Agrotis ypsilon and Spodoptera littoralis), Anz Shadl Pflanz Umwelt, 68:15-17, 1995.

15. Wallner WE, Dubois NR and Grinberg PS : Alternation of parasitism by Rogas lymatriae (Hymenoptera: Braconidae) in Bacillus thuringiensis stressed gypsy moth (Lepidoptera: Lymantriidae) hosts. J Econ Entomol, 76:275-277, 1983.

16. Wallam JD and Yendol WG : Evaluation of Bacillus thuringiensis and a parasitoid for suppression of the gypsy moth. J Econ Entomol, 69:113-118, 1976.

17. Willcocks FC and Bahgat S : The insects and related pests of Egypt. I. part 2. Insects and mites injurious to the cotton plants, published by the Royal Agricultural Soc, pp 406-458, 1937.

18. Sneh B and Gross S : Biological control of the Egyptian cotton leaf worm Spodoptera littoralis (Boisd) (Lep, Noctuidae) in cotton and alfalfa fields using a preparation of Bacillus thuringiensis sp. entomocidus, supplemented with adjuvants. Z Ang Ent, 95:418-424, 1983.

Correspondence: M. Hafez Entomology Department, Faculty of Science, Cairo University, Cairo, EGYPT.