Pakistan J.Sci.Ind.Res., Vol.25, No.5, October 1982

EFFECT OF ENDOTOXIN OF *BACILLUS THURINGIENSIS* ON FECUNDITY AND LONGEVITY OF ADULT BOLLWORM, *HELIOTHIS ARMIGERIA* (Hubn)

Feeroza Khalique, Khalique Ahmad, A. Farid Khan, M. Rafi Shaikh

Department of Microbiology, University of Karachi, Karachi, Pakistan

and

Dilnawaz Shaikh

Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan

(Received Febraury 25, 1981)

This paper presents in detail a comparative account of oviposition results of *Heliothis armigera* (Hubn) adults treated, in larval stage with 10 and 20 µg conc of *Bacillus thuringiensis*-δ-endotoxin spore complexes of HD–1–S–1971 and native strain No. Bt–145. The pre–oviposition period of adults treated* with 10 and 20 µg conc of toxin of HD–1–S–1971 and 10 µg conc of toxin of Bt–145 reduced. The fecundity and longevity of adults treated with 10 and 20 µg conc of spore-δ endotoxin complexes of Bt–145 and 10 µg conc of HD–1–S–1971 reduced significantly. All oviposition experiments were carried out at temperature ranging from 77°–97°F. This paper also includes the description of lamp glass mating–oviposition cage.

INTRODUCTION

The objective of insect pest control through microbial or chemical insecticides is to bring down the pest population to such a level that the insect pest must not be able to cause any significant damage to render the crop economically non feasible or profitable.

Nevertheless *Bacillus thuringiensis* is well known pathogen of lepidopterous larvae and its preparation in the form of microbial insecticides have been proved to be very effective particularly in control of lepidopterous pests. It is understood that these preparations are enjoying wider use due to their effectiveness in hitting specific target and causing no pollution.

It seems to be a fact that when an insect population is allowed to feed on the diet, containing bacterial toxin, certain segments of its population may take smaller amount of bacterial toxins and thus they may escape death or some members of the population happen to be too resistant toward the toxins that they succeed in reaching the adult stage. But such adults which develop from the larvae treated with bacterial toxin may exhibit abnormalities with respect to their physical structure or behaviour resulting in less fecundity and longevity of the adults. Although a few workers conducted studies and traced out the effect of bacterial toxin on the reproductive potential of insects, Dulmage and Martinaz[1] reported the effect of continuous exposure to low concentrations of the δ-endotoxin of *Bacillus thuringiensis* on the development of the Tobacco budworm, *Heliothis virescens.* Abdullah and Abdul–Nasr[2,3] studied the effect of *Bacillus thuringiensis* on reproductive potential of the cotton leafworm, *Spodptera littoralis* (Boisd).

The major objective of this paper is to observe the long term effect of spore–δ-endotoxin complexes of Bt–145 (isolated in Pakistan) and USDA's preparation HD–1–S–1971, on the adult life of *Heliothis armigera* (Hubn) developed from larvae treated with 10 and 20 µg conc. of toxins/ml of the diet.

MATERIAL AND METHOD

Two different preparations of *Bacillus thuringiensis* were used in experimentation. Preparation No. 1 contained spore–δ–endotoxin complex isolated from Bt–145 (isolated in Pakistan) and preparation No. 2 included spore–δ–endotoxin complex of USDA's standard formulation of Bt. labelled HD–1–S–1971. First stage larvae of *Heliothis armigera* procured from the mass rearing programme were used as the test insect. These larvae were individually transferred into capsule vials (1 larva/vial) containing diet mixed with 10 and 20 µg/ml concentrations of the toxins (four batches of infested vials were maintained according to the two concentrations of both the toxins). A batch of infested vials containing diet with no toxin was run as

*Adults treated or treated adults means the adults developed from larvae treated with respective concentrations of the toxin.

control parallel to the tests. All larvae which escaped death in all the treated batches were allowed to feed on the diet containing the respective concentration of toxin till pupation was achieved in the vials. The pupae were transferred in wide mouth glass jar until emergence. The control batch was handled in the same manner.

The adults emerged from control batch and those treated with the respective concentration of the toxins were sex–wise separated and caged in single pair lamp glass mating–oviposition cage (1 pair/cage). All single pair oviposition experiments were conducted through lamp glass mating–oviposition cage which was made as follows:

Lamp Glass Mating–Oviposition Cage (Fig. 1). A medium sized, 10.2 cm high lamp glass (A) of 7.9 cm lower end diameter and 6.6 cm upper end diameter was fashioned into an oviposition cage which held adults for mating and oviposition. Both open ends of the lamp glass were closed with nylon net (B) held in position by scotch tape. A small hole in the centre of upper end of nylon net was made (C) so that the hole–bearing end of the lamp glass becomes top of the cage while the end opposite to it was the bottom. The bottom of the cage was placed on a 9.5 x 1.5 cm petri–dish (E) containing 2.5 cm thick absorbent cotton wool pad (F) serving as oviposition site.

Single pair of adult was released into the cage through the nylon net hole. Another oviposition pad like that of the bottom was prepared and placed on top of the cage covering it with another petri–dish (D). The top and bottom petri–dishes were fixed tightly to the lamp glass by rubber bands. Absorbent cotton wool oviposition pads were daily removed and checked. The adults were daily fed by two absorbent cotton wool pads soaked with 10 % sucrose solution, the diameter of the pads being equal to the top and bottom of the lamp glass. One pad was put in the lower petri–dish in such a way that the entire bottom nylon net came in contact with the soaked pad. Similarly the other soaked pad was kept on the top net which not only provided the feeding surface, but also closed the releasing hole. The adults were allowed to feed for approximately 2 hr. After removing the feeding pads the hole was closed by cotton wool plug and the cage left for about 20–30 min in order to dry the nylon net surface. Then the cage was again provided with new oviposition pads for next day oviposition.

All single pair oviposition experiments continued till the death of the female.

RESULTS AND DISCUSSION

Effect of 10 and 20 µg Conc of Toxin of HD–1–S–1971 on Adults Longevity and Fecundity. The results given in the Table 1 indicated that the average pre–oviposition period, total eggs/female and the longevity of adults developed from control group, were 3.583 days, 553.000 eggs/female and 17.166 days respectively. –

Fig. 1. Lamp glass mating ovi-position cage. (A) Lamp glass. (B) Nylon net. (C) Hole in the nylon net. (D) and (E) Petri dishes. (F) Cotton wool pad. Below is assembled cage.

A comparison of results between control and those adults treated with 10 µg conc of toxin/ml of diet, showed that there occurred no marked difference in pre–oviposition period, while a significant reduction of 324.000 eggs/female was observed. The female longevity was also decreased by 2.566 days. Similarly, in case of 20 µg conc treated adults, no significant difference was noted in pre–oviposition period as compared to 10 µg conc treated adults. The fecundity was reduced by 213.572 eggs/female and longevity remained nearly the same as that observed in control adults. Dulmage and Martinez[1] reported that the female from larvae reared on diet containing 10.0 IU of toxin/ml diet, laid few eggs. Abdullah and Abdul–Nasr [2, 3] also reported decline in the average number of eggs per female and decrease in longevity of adult females. They also observed that the low fecundity and longevity of the treated female depended on the length of toxin feeding period during the larval stage.

A conclusion can be drawn from the results of adults treated with 10 and 20 µg conc of HD–1–S–1971 that the feeding of spore–δ-endotoxin complex throughout the larval period of this insect resulted in the significant reduction in the reproductive potential.

The phenomenon of reduction in fecundity and longevity of treated adults may be attributable to some physical and/or physiological abnormality that may have resulted due to infection of the pathogen caused by feeding of the toxin.

Effect of 10 and 20 µg Conc of Toxin of Bt–145 on Adult's Longevity and Fecundity. The results illustrated

Table 1. Effect of *Bacillus thuringiensis* δ-endotoxin on the adult longevity and fecundity of Bollworm, *Heliothis armigera* (Hubn).

Level of toxin conc/ml diet.	No. of pairs	Average		
		Preoviposition period*. (days)	Total No. of eggs per female*.	Longevity of female*. (days)
Control	12	3.583±0.951	553.000±295.393	17.166±4.600
HD–1–S–1971 10 µg/ml.	10	3.100±2.776	229.000±194.543	14.600±3.309
Bt–145 10 µg/ml.	14	2.714±1.282	244.571± 54.411	14.571±2.658
HD–1–S–1971 20 µg/ml.	7	3.57 ±0.990	339.428±232.087	17.071±3.775
Bt–145 20 µg/ml.	4	4.250±1.732	256.500±263.000	12.500±9.961

Temperature range: 77°F – 97°F; * Including 95% confidence limit.

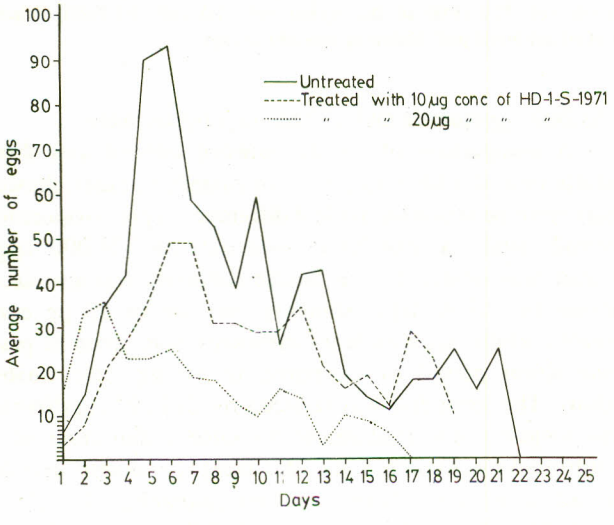

Fig. 1.A. Average ovi-position rate of H. armigera (Hubn) adults treated with 10 and 20 ug cone of HD – 1– S. 1971.

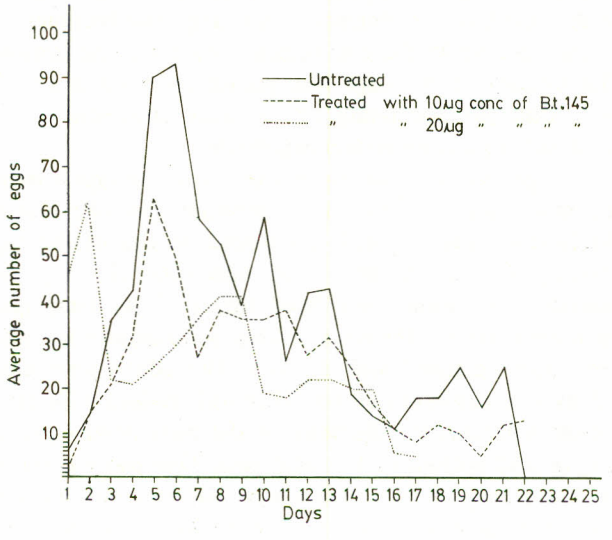

Fig. 1. B. Average ovi-position rate of H. armigera (Hubn) adults treated with 10 and 20 ug cone of B. T. – 145.

in the Table 1 indicated that there occurred a decrease in pre–oviposition period, number of eggs/female and longevity of female adults treated with 10 µg conc. Pre–oviposition periods of adults treated with 20 ug conc was not significantly increased as compared with control. But the number of eggs/female and the longevity of the adults treated with 10 and 20 µg conc of toxin significantly reduced by 308.429 eggs and 296.500 eggs/female and 2.595 days and 4.666 days respectively as compared with control.

It is evident from the results that the reproductive potential of the female moth was observed to be adversely affected due to feeding of the toxin in larval stage.

Average Oviposition Rate of Treated & Untreated Adults (Figs. 1A and 1B). Fig. 1A indicates the pattern of oviposition of adults treated with 10 and 20 µg conc of toxin of HD–1–S–1971 and those of untreated adults. The oviposition pattern of untreated insect showed a gradual increase and reaching the maximum egg yield on 6th day. Thereafter the oviposition rate of untreated insects gradually dropped to zero until death of the female on 22nd day with intermittent rise and fall in egg laying. In 10 µg conc treated adults the oviposition reached its peak on 6th and 7th day, but the number of eggs significantly dropped by one half than the untreated, and ultimately the oviposition came to an end on 19th day with gradual increase and decrease. Similarly in 20 µg conc treated insects the rate of oviposition further reduced with intermittant rise and fall and coming to an end on 17th day.

Fig. 1B illustrated the oviposition rate of adults treated

with 10 and 20 µg conc of toxin of Bt–145. In 10 µg conc treated insects, the maximum eggs were obtained on 5th day of oviposition, later significant reduction in egg laying was noted and gradually dropped and ended on 22nd day with intermittant rise and fall. In 20 µg conc treated adults, the egg laying peak was observed on 2nd day of egg laying and the oviposition came to an end on 17th day.

It can be concluded from the results given in the Figs. 1A and 1B that the oviposition of insects treated with 10 and 20 µg conc of both the toxins reduced significantly as compared to untreated insects. Therefore it became quite evident from the results that the feeding of δ–endotoxin spore complexes of HD–1–S–1971 and Bt–145 seems to have an adverse effect on the fecundity of the treated insects.

Abdullah and Abdul–Nasr[2, 3] reported that the marked reduction, delay short period and irregularity are the most conspicuous features of the treated insects. However, Dulmage and Martinez[1] reported that the low fertility resulted from the poor physical condition of the surviving females rather than from any direct effect of the toxin on the sexual process.

CONCLUSION

It became clear from the results and discussion that the application of spore–δ–endotoxin complexes of both the formulations of HD–1–S–1971 and Bt–145 certainly exhibited long term effect with respect to marked reduction in oviposition and shortening of the moth life span.

Acknowledgement. This research has been financed in part by grant (FG–Pa–317, PK–ARS–146) made by the United States Department of Agriculture, Agricultural Research Service, under PL–480 programme.

REFERENCES

1. H.T. Dulmage and E. Matinez, J. Invertab. Pathol., 22, 14 (1973).
2. Abdullah and Abul–Nasr, Bull. Ent. Soc. Egypt, Econ. Ser.IV, 171–176 (1970).
3. Abul-Nasr and Abdullah, Bull. Ent., Soc. Egypt, Econ. Ser.IV, 151–160 (1970).