http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Science, 6 (6): 615-621, 2003
ISSN 1028-8880

Impact of *Bacillus thuringiensis* Subsp. *Kurstaki* on Biology of *Helicoverpa armigera*

Feeroza Khalique and Khalique Ahmed
Pulses Programme, Institute of field and Horticultural Crops,
National Agricultural Research Center, National Park Road, Islamabad, Pakistan

Abstract: Studies on mortality and rate of development of *Helicoverpa armigera* due to feeding of spore-δ-endotoxin complex of indigenous strain HD-695 (8,500 IU mg^{-1}) and standard strain HD-1-S-1980 (16,000 IU mg^{-1}) of *Bacillus thuringiensis* var. *kurstaki* revealed that the differences in potencies did not cause significant differences in retardation. Both the bacterial toxins induced similar biological effects (significant larval mortalities, marked larval retardation, reduction in pupation, emergence and fecundity, as well as inconsistent increase in pre-oviposition period and prolongation of generation period) of the test insect.

Key words: *Bacillus thuringiensis, Helicoverpa (Heliothis) armigera,* spore-δ-endotoxin, biology

Introduction

Insect pests are a major cause of damage to the world's commercially important agricultural crops. *Helicoverpa armigera* (Huebner) is one of the most devastating insect pest of many crops. Indiscriminate, continuous and excessive use of chemical insecticides have created a number of problems i.e., development of resistance in pest population biological and environmental hazards and water pollution etc. It is, therefore imperative on part of the agricultural scientists to use and recommend only those insecticides having no or least biological hazards. *Bacillus thuringiensis* Berliner (*B.t.*) is a naturally occurring, gram-positive, spore-forming soil-bacterium and preparations containing *B.t* are widely used as a biological insecticide in agriculture and forestry. (Deml *et al*., 1999). Owing to its specific mode of action, *B.t.* products are unlikely to pose any biological and environmental hazards (Anonymous, 1999). In order to evaluate properly the role of pathogen (*B.t.*) in management of insect pest, the study should be extend to trace the effect of *B.t.* on the survivors. Among the valuable contributions pertaining to this subject are those of Yamvrias (1962), Abdallah and Abul-Nasr (1970 a and b), Morris (1977), Dulmage *et al.* (1978), Khalique *et al.* (1982), Fast and Regniere (1984), Retnakaran *et al.* (1983), van Frankenhuzen (1987), Ajanta *et al.* (1999) Deml *et al.* (1999) and Khalique and Ahmed (2001and 2002). Present study elaborates on the impact of spore-δ-endotoxin complex of indigenous strain (HD-695) of *Bacillus thuringiensis* and its comparison with spore-crystal complex of standard reference strain (HD-1-S-1980) on the mortality and biology of *Helicoverpa armigera*.

Materials and Methods

Rearing of the test insect, *H. armigera* was done on the artificial diet formula developed by Ahmed 1983 and neonate (1st instar) larvae were used in the bioassay. Two strains of *Bacillus thuringiensis* subsp. *kurstaki*, the indigenous strain namely HD-695 and reference standard strain HD-1-S-1980 having predetermined potency 8,500 IU mg^{-1} (Khalique and Ahmed, 2001) and 16,000 IU mg^{-1} (arbitrary potency and Versoi, 1981) respectively were used in the experiments.

Nine serial dilutions of both the strains were prepared in buffered solution pH 7.0 (Khalique and Ahmed, 2002). Ten ml of *B. thuringiensis* buffer suspension from No.1 was pipetted out and added to 90.0 ml of freshly prepared diet kept at 55-60°C in 250 ml beaker. This mixture was thoroughly mixed to obtain resulting concentration of 60.0 μg ml^{-1} diet. The rest of the toxin concentration from 45.0, to 3.75 μg ml^{-1} diet were prepared from suspension number 2 to 9, respectively.

After thorough mixing of the toxin into diet, the mixture was distributed into twenty five sterilized capsule vials so that each capsule vial contained approximately 4.0 ml of toxin mixed diet. In control only 10.0 ml buffer was added. The capsule vials containing treated and untreated diet were kept at room temperature for about 30 minutes for cooling and solidification of diet.

Each vial containing toxin mixed diet and without toxin (control) was infested with one neonate larva with the help of a sterilized soft camel hair brush. A separate brush was used for each concentration and control group. The infested vials were plugged with sterilized cotton wool. The capsule vials were kept upside down at room temperature, which ranged from 24±2.5°C and 65 to 85% RH. Each experiment was replicated four times in different days and 25 larvae were used for each replicate.

In these experiments, larvae were allowed to feed on spore-δ-endotoxin mixed diet for ten days and then transferred to toxin free diet and allowed to developed till

pupation. To avoid any influence of the diet causing possible deterioration over long period of larval development, the fresh diet was placed in the capsule vials from time to time. Records were maintained on daily basis to determine larval mortality, larval period, pupation, pupal period and emergence of adults. All pupae from each treatment and control batches were weighed within 24 h of pupation and placed on a blotting paper in a wide mouth glass jar for adult emergence. The data on longevity of emerged adult, pre-oviposition period, sex ratio and fecundity of adult were also recorded. Normal adults from treated and untreated group were paired separately and released in glass lamp oviposition cages (2 pair/cage) (Khalique *et al*., 1982) and the cages were checked on alternate days till death of the pairs.

Results

Cumulative mortalities: The concentration-mortality response of *H. armigera* at various developmental stages for ten-days exposure to HD-695 and HD-1-S-1980 indicated (Table 1 and 2) that maximum mortalities were obtained within seven days. Further increase in mortalities between 7 days and pupation remained within the range of 3.0 to 13.0% for both the toxins in different concentrations. There was no pronounced difference in mortalities at pupation and at emergence for both the toxins. The mortalities recorded in control (common to both the strains) were 0.0, 1.0 and 10.0% at 7 days, at pupation and at emergence, respectively. (Table 1 and 2).

Effect of toxin on larval development and pupation: The average larval period of control larvae was 23.3±0.46 days (Table 3). On comparison with control, the average larval periods at the lowest concentration (3.75 μg ml^{-1}) of HD-695 and HD-1-S-1980 were recorded to be 28.01±0.61 days and 27.13±0.68 days, respectively, indicating a marked difference between untreated and treated larvae. As the concentrations of the toxins increased, the larval time also increased. No marked difference in larval retardation was noted due to feeding of HD-695 and HD-1-S-1980. The highest retardation (34.6±2.76) in larval development was noted at 60.00 μg ml^{-1} of HD-695. The percentage of the larvae that survived and pupated was reduced to 6.0 and 5.0% for those larvae reared on diet containing 60.0 μg toxin ml^{-1} diet of HD-695 and HD-1-S-1980 respectively, as compared with 99.0% in the control. In both the strains, the percentage of larvae s ucceeded t o reach pupation,

Table 1: Concentration-mortality response of *H. armigera* at different developmental stages after exposure to spore-δ-endotoxin of HD-695

Level of toxin μg ml^{-1} of diet	Mortality* %±SD		
	7 days	At pupation	At Emergence
3.75	21.2±13.61	24.2±11.76	22.1±15.09
5.625	24.0±5.66	35.3±6.51	38.8±5.84
7.5	44.0±9.80	50.5±11.41	49.9±11.44
11.25	44.0±5.66	52.5±14.07	49.9±14.84
15.0	60.2±5.72	66.0±6.58	63.7±7.27
22.5	61.0±9.45	70.7±8.22	68.9±6.24
30.0	73.0±10.0	85.8±7.66	84.4±9.44
45.0	77.0±3.83	90.9±6.09	91.1±6.48
60.0	85.0±8.25	93.9±5.16	93.9± 5.38

Table 2: Concentration-mortality response of *H. armigera* at different developmental stages after exposure to spore-δ-endotoxin of HD-1-S-1980

Level of toxin μg ml^{-1} of diet	Mortality* %±SD		
	7 days	At pupation	At Emergence
3.75	40.0 ±13.47	45.4 ±11.76	45.5 ±18.11
5.625	45.0 ±16.77	50.5 ±19.69	51.1 ±17.04
7.50	50.0 ±13.66	61.6 ±12.24	61.1 ±14.52
11.25	62.0 ±12.44	72.7 ±13.03	72.2 ±15.32
15.0	68.2 ±04.62	75.7 ±02.95	75.5 ±01.47
22.5	67.0 ±11.02	80.8 ±12.72	84.4 ±08.60
30.0	81.0 ±11.49	90.9 ±13.40	91.1 ±13.00
45.0	84.0 ±00.00	89.9 ±02.23	88.9 ±02.26
60.0	88.0 ±05.66	94.9 ±05.02	94.4 ±05.45

* Mortalities were corrected for the natural response rate (Abbott, 1925)

decreased with the increase in toxin concentration. (Table 3).

Effect of toxins on pupal weight, pupal duration and emergence: The average weight of pupae developed from untreated larvae was 0.377±0.090 g, (Table 4) while the average weight of pupae developed from those treated with different concentrations of HD-695 varied from 0.371±0.010 g to 0.384±0.057 g, indicating negligible difference in pupal weight among various concentrations. The average weight of the pupae developed from larvae treated with different concentrations of HD-1-S-1980 also varied from 0.362±0.051 g to 0.387±0.011 g. In general the weight of pupae developed from treated larvae was found more or less similar than that of untreated ones, showing no adverse effect of the toxins on the pupal weight. Overall the percentage of adult emergence was also found to be affected as it was 49 and 70% after larval exposure to the toxin of HD-1-S-1980 and HD-695 respectively at 3.75 μg ml^{-1} and decreased up to 4% at 60 μg toxin ml^{-1} of diet in both the strains, as compared to control emergence (90%).

Table 3: Influence of spore-δ-endotoxin of HD-1-S 1980 and HD-695 on the larval development *Helicoverpa armigera*

	HD-1-S-1980			HD-695		
Level of toxin (μg ml^{-1} diet)	No. of survivors/total	Average* larval Period (days) ±SD	Pupation (%)	No. of survivors/total	Average* larval Period (days) ±SD	Pupation (%)
0.00	99/100	23.3±0.46	99.0	99/100	23.3±0.46	99.0
3.75	54/100	27.1±0.68	54.0	75/100	28.0±0.60	75.0
5.625	49/100	29.0±0.96	49.0	64/100	29.6±0.85	64.0
7.5	38/100	28.8±0.90	38.0	49/100	29.8±1.04	49.0
11.25	27/100	29.5±1.18	27.0	47/100	28.8±0.98	47.0
15.0	24/100	30.9±1.56	24.0	33/100	30.2±1.02	33.0
22.5	19/100	31.3±1.86	19.0	29/100	31.1±1.19	29.0
30.0	9/100	32.0±2.88	9.0	14/100	31.5±1.72	14.0
45.0	10/100	32.2±1.49	10.0	9/100	31.4±0.57	9.0
60.0	5/100	31.4±0.96	5.0	6/100	34.6±2.76	6.0

Table 4: Influence of spore- δ- endotoxin of HD-1-S 1980 and HD-695 on the pupal weight of *Helicoverpa armigera*

	HD-1-S-1980		HD-695	
Level of toxin (ug ml^{-1} diet)	No. of survivors/total	Average* pupal weight (g)±SD	No of survivors/ total	Average * pupal weight (g)±SD
0.00	97/100	0.377±0.090	97/100	0.377±0.090
3.75	52/100	0.387±0.011	75/100	0.371±0.010
5.625	48/100	0.379±0.011	60/100	0.371±0.011
7.5	37/100	0.376±0.012	48/100	0.378±0.014
11.25	27/100	0.386±0.015	47/100	0.374±0.012
15.0	23/100	0.371±0.015	33/100	0.379±0.012
22.5	18/100	0.385±0.028	29/100	0.376±0.016
30.0	8/100	0.362±0.051	14/100	0.367±0.024
45.0	10/100	0.372±0.025	8/100	0.380±0.032
60.0	5/100	0.373±0.060	6/100	0.384±0.057

*Including 95% confidence limit

The average pupal duration of untreated and treated group of HD-695 and HD-1-S-1980 (Table 5) showed that the average pupal period of control pupae was 17.23±0.37 days. A comparison of average pupal period of control pupae with those treated with various concentrations of HD-695, indicated that pupal period increased slightly, at the highest concentration (60 μg ml^{-1}), the average pupal period was 19.00±1.63 days. Similarly a slight increase in pupal duration of the pupae obtained from different treatments of HD-1-S-1980 was also noted. The results indicated that irrespective of the difference in potencies, no marked effect appeared in pupal weight and pupal duration of the insect. (Table 4 and 5).

Effect of toxins on the sex ratio and pre-oviposition period: The results of the control group illustrated that the natural sex ratio was almost 1:1 (Table 6). A comparison of sex ratios of control adults with those of the treated ones indicated that spore-crystal-complex of HD-695 and HD-1-S-1980 had no significant effect on sex ratio but a slight deviation from the natural sex ratio was found at 3.5, 7.5 and 11.25 μg ml^{-1} of HD-1-S-1980. The average pre-oviposition period of control adults was 3.66±0.14 days. The results of treated group (HD-695) when compared to untreated one, showed that there occurred an irregular increase in pre-oviposition period in different concentrations of toxin. Similar results were obtained in pre-oviposition period of HD-1-S-1980 treated group with maximum period (8.42 days) in 15.0 μg ml^{-1} treatment. No significant difference was noted in pre-oviposition periods of adults treated with HD-695 and HD-1-S-1980. (Table 6).

Effect of toxins on longevity and fecundity of adults: The average longevity and fecundity of treated and untreated adults are given in Table 7. The average longevities of male and female adults in control group were 21.8±4.88 days and 18.30±3.31 days, respectively. No adverse effect in longevities was observed between the untreated and toxins (HD-1-S-1980 and HD-695) treated adults.

The average fecundity of adults in control was 969.25±255.81, while the fecundities of adults emerged from 3.75 μg ml^{-1} treatment of HD-695 and HD-1-S-1980 were 772.30±293.26 and 730.10±70.66 respectively. These results indicated that there was a marked difference in fecundity of treated and untreated adults. When the average fecundities of adults were compared among the treatments of HD-695 and HD-1-S-1980, there was no consistent response of the insect between the treatments and the reproductive potential of the treated adults. (Table 7).

Effect of toxin on survival time: The time of death of *H. armigera* larvae were observed at different intervals after

Table 5: Influence of spore-δ- endotoxin of HD-1-S 1980 and HD-695 on the pupal duration of *Helicoverpa armigera*

	HD-1-S-1980			HD-695		
Level of toxin (μg ml^{-1} diet)	No. of survivors/total	Average* pupal period (days)±SD	Emergence (%)	No. of survivors/total	Average* pupal period (days)±SD	Emergence (%)
0.00	90/100	17.23±0.37	90.0	90/100	17.23±0.37	90.0
3.75	49/100	18.20±0.51	49.0	70/100	17.61±0.45	70.0
5.625	44/100	19.18±0.69	44.0	50/100	17.94±0.47	50.0
7.50	35/100	18.77±0.68	35.0	45/100	17.80±0.70	45.0
11.25	25/100	18.68±1.11	25.0	47/100	18.10±0.66	47.0
15.00	22/100	18.40±0.84	22.0	32/100	17.87±0.65	32.0
22.50	14/100	19.21±0.65	14.0	28/100	18.17±0.70	28.0
30.0	7/100	18.86±0.90	7.0	14/100	18.28±1.22	14.0
45.0	10/100	18.70±1.07	10.0	8/100	18.37±1.08	8.0
60.0	4/100	18.0±3.18	4.0	6/100	19.00±1.63	4.0

Table 6: Influence of spore- δ- endotoxin of HD-1-S 1980 and HD-695 on the sex ratio and pre-oviposition period of *Helicoverpa armigera*

	HD-1-S- 1980		HD-695	
Level of toxin (μg ml^{-1} diet)	Sex Ratio M : F	Av*. Pre-oviposition periods (days ± SD)	Sex Ratio M : F	Av*. Pre-oviposition periods (days ± SD)
0.00	1:1	3.66±0.14	1:1	3.66±0.14
3.75	1:1.4	4.90±1.30	1:1.2	3.83±0.35
5.625	1:1	5.00±1.44	1:1	5.00±1.80
7.5	1:1.5	5.87±1.84	1:1.1	6.22±2.92
11.25	1:0.67	5.63±0.63	1:1	4.70±0.96
15.0	1:1	8.42±0.99	1:1.1	5.36±1.22
22.5	1:1	5.33±1.53	1:0.8	6.14±3.50

Table 7: Impact of spor-δ-endotoxin complex of HD-1-S-1980 and HD-695 on the longevity and fecundity of *Helicoverpa armigera* (Hubn)

	HD-1-S-1980			HD-695		
	Av.* longevity of adult (days) ± SD		Av.* fecundity of adult	Av.* longevity of adult (days) ± SD		Av*. fecundity of adult
Level of toxin (μg ml^{-1} diet)	M	F	(days) ± SD	M	F	(days) ± SD
0.0	21.8±4.9	18.3±3.3	969.3±255.8	21.8±4.9	18.3±3.3	969.3±255.8
3.75	22.2±5.8	18.7±4.7	730.1±70.7	20.5±3.6	19.1±4.1	772.3±239.3
5.625	19.0±6.1	17.4±4.0	566.8±307.5	19.5±3.8	15.9±1.7	780.0±68.7
7.50	18.0±5.6	16.8±2.7	745.6±276.5	19.4±5.2	17.3±4.4	648.7±137.8
11.25	20.4±4.9	19.9±6.7	794.0±252.3	16.5±4.4	18.2±3.8	483.2±145.5
15.00	16.7±5.5	19.7±6.7	395.0±218.9	20.7±5.5	18.7±4.6	664.2±299.8
22.5	22.5±2.3	14.8±6.9	693.3±393.7	19.1±9.4	16.4±6.8	467.7±317.2

* Including 95% confidence limit

Table 8: Time of death of *Helicoverpa armigera* (Hubn) larvae exposed to spor-δ-endotoxin complex of HD-1-S-1980 and HD-695

		HD-1-S-1980			HD-695	
		Mortality % during indicated period (days)			Mortality % during indicated period (days)	
Level of toxin (μg/ ml diet)	No. of death/Total	0-10	11-35	No. of death/Total	0-10	11-35
0.0	1/100	0	100.0	1/100	0.0	100
3.75	45/100	97.8	2.2	23/100	95.7	4.3
5.625	52/100	96.2	3.8	36/100	91.7	8.3
7.50	62/100	90.3	9.7	51/100	98.0	2.0
11.25	73/100	98.6	1.4	53/100	96.2	3.8
15.00	75/100	96.0	4.0	65/100	98.5	1.5
22.5	81/100	96.3	3.7	71/100	97.2	2.8
30.0	91/100	97.8	1.2	86/100	97.7	2.3
45.0	90/100	97.8	1.2	91/100	97.8	2.2
60.0	95/100	100	0.0	94/100	100	0.0

exposing the larvae for 10 days to spore-δ-endotoxin complex of HD-695 and HD-1-S-1980. Both toxins caused larval mortalities in the first ten days of the exposure (Table 8). The larvae that survived after 10 days of toxin exposure showed very low mortality rate in the rest of the period (11-35 days) (Table 8).

Discussion

The concentration-mortality response of *H. armigera* at different development stages indicated maximum mortalities with in 7 days. These results are in agreement with Ali and Young (1993) they reported most mortality (60-91%) of *Helicoverpa zea* and *Heliothis virescens* occurred at 7 days and pupation. In the field survival of both the species generally decreased as *Bacillus thuringiensis* rate increased.

The results on larval developmental period showed that all treatments of both the toxins adversely affected the larval development by causing significant delay. Alchanatis *et al.* (2000) used an image analysis system to test the effect of feeding *Bacillus thuringiensis* (Bt) transgenic cotton to *H. armigera* larvae; it reduced leaf consumption h-1 and increased the inter-meal time. This feeding profile suggests that *Bt* toxic protein suppressed feeding, because the longer resting times than those of controls were probably needed by the larvae to recover from the *Bt* toxicity. Karim *et al.* (2000) showed a correlation between toxin concentration and inhibitory response with Cry2A toxin and the lag time decreased with increasing concentration of toxin applied, which is evidence of dose response. The delayment of larval period corresponds to observations of Khalique and Ahmed (2001), they reported that increase in larval period (after 7 days exposure) was directly related to concentration of toxin present in the diet. No marked differences in larval retardation was noted between the survivors of HD-1-S-1980 and HD-695 treatments thereby indicating that the difference in potencies of these toxins did not contribute to any significant difference in larval period. These results also strengthenened by the earlier observations of Khalique and Ahmed (2002), they reported non-significant differences between the larval period of *H. armigera* exposed to strains (HD-1-S-1980 and HD-244) for seven days.

The average pupal weight of HD-695 treated group did not differ from the average weight of pupae reared on HD-I-S- 1980 toxin and there was also no difference in pupal weight of untreated and treated pupae. These results also fell in agreement with the observation recorded in the context of the seven days exposure effect of HD-694 (Khalique and Ahmed, 2001); HD-244 and HD-1-S-1980 (Khalique and Ahmed, 2002) on pupal weight indicating that *H. armigera* larvae have pronounced ability to recover from ten days exposure of *B. thuringiensis* and could pupate normally. The observations supported the results of Abdallah and Abul-Nasr (1970b), Fast and Regniere (1984) and Dulmage *et al.* (1978).

The average pupal duration of HD-695 and HD-1-S-1980 treated group showed non-significant difference after 10 days exposure, such observation were also reported by Soliman *et al.* (1970), Dulmage and Martinez (1978), Dulmage *et al.* (1978) and Salama *et al.* (1981). Cui and Xia (1999) studied the effects of transgenic B.t. cotton on development and reproduction of *Helicoverpa armigera*. They observed that rate of pupation and emergence decreased by 48.2-87.5 and 66.7-100%, respectively. The difference in potencies of these strains did not show any impact on pupal duration and emergence. Justin *et al.* (1994) evaluated the interaction of *Bacillus thuringiensis* Berliner with NPV against *H. armigera*, they found an inverse co-relation between adult emergence with the dose of *B.t.* No marked difference due to bacterial toxins in sex ratio was observed other than slight deviation of sex ratio from the natural pattern in some treatments (3.75 and 7.50 μg ml^{-1} of HD-1-S-1980). Similar results were reported by Afify and Matter (1969) that out of three treatments one treatment showed a slight decrease in the ratio of females but gave no evidence of a definite effect of the pathogen on the ratio. McGaughy (1978) reported that *B. thuringiensis* (Dipel) formulation had no appreciable effects on the sex ratio of moths, *E. cautella* and *P. interpunctella* that survived. Soliman *et al.* (1970) reported that sex ratio was not changed to the side of females. The effect of toxins on the sex ratio did not significantly differ from each other. The observation of Morris (1976) holds good in support of present results with non-significant difference in sex ratio of *C. fumiferana* treated with Thuricide and Dipel.

The average pre-oviposition period of the adults developed from larvae treated with HD-695 and HD-1-S-1980 steadily increased in comparison with control. In case of HD-695, the increase in pre-oviposition period at higher concentrations was more pronounced than that at lower concentrations and similar trend of increase in pre-oviposition was observed in case of HD-1-S-1980. Afify and Matter (1969) reported non-significant difference in the pre-oviposition period between the control and the treated *A. kühniella*.

Abdallah and Abul-Nasr (1970a) observed delay and irregularity in oviposition of *S. littoralis* adults when larvae were allowed to feed on Biotrol sprayed cotton leaves at a concentration of 2.0% for different periods. They also observed that shortening of the moth life span was consistent feature of the treatment of *B. thuringiensis*. Salama *et al.* (1981) illustrated that the longevity of the moth of *H. armigera* and *S. liltoralis* was not affected as a result of larval treatment and showed no clear correlation with concentration of larval exposure to the toxin.

Present results indicated no considerable difference in longevity of HD-695 and HD-1-S-1980 treated groups at all

concentrations in comparison with control. The substantial reduction, delay and irregularity in oviposition of *Spodoptera littoralis* due *B. thuringiensis* treatment was reported by Abdallah and Abul-nasr (1970a). A significant reduction in the fecundity of *A. kuehniella*, after exposure of *B. thuringiensis* was also observed by Afify and Matter (1969). The longevities of both the groups also differed non-significantly with each other. A comparison of control fecundity with those of HD-695 and HD-1-S-1980 treated batches showed considerable but inconsistent reduction in fecundity at almost all the *B. thuringiensis* treatments. Soliman *et al.* (1970) reported variations in longevity are of no significance with regard to the population density, as long as the sex ratio is not changed to the side of the females and the fertility of the females is adversely affected with larval treatment.

The results of the ten days exposure of the toxins (HD-695 and HD-1-S-1980) revealed that larval treatment mainly resulted in causing retardation in larval development period, decreasing pupation percentage and adult emergence, increase in pre-oviposition period, decrease in fecundity, no significant effect on pupal weight and pupal duration. The comparative study of two toxins (HD-695 and HD-1-S-1980) did not significantly differ from each other in causing a marked difference in disorientation in the development of *H. armigera*.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.

Abdallah, M.D. and S. Abul-Nasr, 1970a. Effect of *Bacillus thuringiensis* Berliner on the reproductive potential of the cotton leaf worm. Bull. Ent. Soc. Egypt, Econ. Ser., 4: 171-175.

Abdallah, M.D. and S. Abul-Nasr, 1970b. Feeding behaviour of the cotton leaf worm, *Spodoptera littoralis* (Boisd), Sublethally infected with *Bacillus thuringiensis* (Berliner). Bull. Ent. Soc. Egypt, Econ. Ser., 4: 161-169.

Afify, A.M. and M.M. Matter, 1969. Retarded effect of *Bacillus thuringiensis* Berliner on the fecundity of *Anagasta kuhniella* (Zell.) Entomophaga, 14: 447-456.

Ahmed, K., 1983. Development of artificial diets for mass breeding of some lepidopterous insects. M. Phil Thesis, Dept. Zool. Univ. Karachi, Pakistan.

Ajanta, C., N.C. Kaushik, G.P. Gupta and A. Chandra, 1999. Studies of *Bacillus* on growth and development of *Helicoverpa armigera* (Huebner). Ann. Plant Prot. Sci., 7: 154-158.

Alchanatis, V., A. Navon, I. Glazer and S. Levski, 2000. An image analysis system for measuring insect feeding effects caused by biopesticides. J. Agril. Engineering Res., 77: 289-96.

Ali, A. and Sy. Young, 1993. *Bacillus thuringiensis* var *kurstaki* activity against larvae of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae) on cotton. J. Econ. Entomol., 86: 1064-1068.

Anonymous, 1999. *Bacillus thuringiensis*. Environmental Health Criteria. International Programme of chemical Safety. World Health Organization.

Cui, J.J. and J.Y. Xia, 1999. Effects of Transgenic Bt cotton on development and reproduction of the cotton bollworm. Acta Agric. Univ. Henan., 33: 20-24.

Deml, R., T. Meise and K. Detner, 1999. Effects of *Bacillus thuringiensis* o-endotoxin on food utilization, growth and survival of selected phytophagus insects. J. Appl. Ent., 123: 55-64.

Dulmage, H.T., H.M. Graham and E. Martinez, 1978. Interaction between the tobacco budworm, *Heliothis virescens* and the δ-endotoxin produced by the HD-1 isolate the *Bacillus thuringiensis* var. *kurstaki*. Relationship between length of exposure to the toxin and survival. J. Invertebr. Pathol., 32: 40-50.

Fast, P.G. and J. Regniere, 1984. Effect of exposure time to *Bacillus thuringiensis* on mortality and recovery of the spruce budworm (Lepidoptera: Tortricidae) Can. Ent., 116: 123-130.

Justin, C.G.L., R.J. Rabindra and S. Jayraj, 1994. Laboratory evaluation on the interaction of *Bacillus thuringiensis* Berliner with NPV against *Helicoverpa armigera* (Hubner). Pest Management and Econ. Zool., 2: 101-103.

Karim, S., F. Gould, DH. Dean and S. Karim, 2000. *Bacillus thuringiensis* delta-endotoxin protein Show a correlation in toxicity and short circuit current inhibition against *Helicoverpa zea*. Current Microbiol., 41: 214-219.

Khalique, F., K. Ahmed. A.F. Khan, M.R. Sheikh and D. Sheikh, 1982a. Effect of spore-δ-endotoxin of *Bacillus thuringiensis* on the development of corn earworm, *Heliothis armigera* (Hübn.). Pak. J. Sci. Ind. Res., 25: 28-30.

Khalique, F., K. Ahmed, A.F. Khan, M.R. Sheikh and D. Sheikh, 1982b. Effect of spore-o-endotoxin of *Bacillus thuringiensis* on fecundity and longevity of adult boll worm, *Heliothis armigera* (Hübn.). Pak. J. Sci. Ind. Res., 25: 180-183.

Khalique, F. and K. Ahmed, 2001. Toxicity of *Bacillus thuringiensis* subsp. *kurstaki* Berliner and sub-lethal effect on development of *Helicoverpa* (*Heliothis*) *armigera* Huebner. Pak. J. Biol. Sci., 4: 529-534.

Khalique, F. and K. Ahmed, 2002. Retarding effect of spore-δ-endotoxin complex of *Bacillus thuringiensis* Berliner strains on development of *Helicoverpa armigera* (Huebner) Pak. J. Biol. Sci., 5: 853-857.

McGaughy, W.H., 1978. Effects of Larval age on the susceptibility of almond moths and Indian meal moths to *Bacillus thuringiensis*. J. Econ. Entomol., 71: 923-925.

Morris, O.N., 1976. A 2-years study of the efficacy of *Bacillus thuringiensis* Chitinase combinations in spruce budworm, *Choristoneura fumiferana* control. Can. Ent., 108: 225-233.

Morris, O.N., 1977. Long term study of the effectiveness of aerial application of *Bacillus thuringiensis* acephate combinations against the spruce budworm, *Choristoneura funiferana* (Lepidoptera: Tortricidae). Can. Ent., 9: 1239-1248.

Retnakaran, A., H. Lauzon and P. Fast, 1983. *Bacillus thuringiensis* induced anorexia in the spruce budworm, *Choristoneura fumiferana*. Ent. Exp. and Appl., 34: 233-239.

Salama, H.S., M. S.Foda, A. Sharaby, M. Matter and M. Khalafallah, 1981. Development of Some Lepidoterous Cotton pests as affected by exposure to sublethal levels of endotoxins of *Bacillus thuringiensis* for different periods. J. Invertebr. Pathol., 38: 220-229.

Soliman, A.A., A.M. Afify, H.A. Abdel-Rahman and A.A. Widad, 1970. Effect of on the biological potency of Pieris rapae (Lep., Pieridae). Z. Agnew.

van Frankenhuzen, K. and W.C. Nystrom, 1987. Effect of temperature on mortality and recovery of spruce budworm (Lepidoptera:Tortricidae) exposed to *Bacillus thuringiensis* Berliner. Can. Ent., 119: 941-954.

Versoi, P.L., 1981. Bioassay of *Bacillus thuringiensis* preparations using cabbage looper or tobacco budworm larvae. Standard technique Report No. 1-80 Entomology Section, Biochem Products Division, Salsbury Laboratories Charles City, Iowa 50616, USA.

Yamvrias, C., 1962. Contribution a L'etude due mod D`action de *Bacillus thuringiensis* Berliner VIS-a-vis de la teigne de la farine *Anagasta kuhniella*. Entomophaga, 7: 101-159.