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 Sciences 5 (8): 853-857, 2002

Retarding Effect of Spore-δ-endotoxin Complex of *Bacillus thuringiensis* (Berliner) Strains on the Development of *Helicoverpa armigera* (Huebner)

Feeroza Khalique and Khalique Ahmed
Pulses Programme, National Agricultural Research Center,
P. O. NIH, National Park Road, Islamabad-45500, Pakistan

Abstract: The retardation in the development of *Helicoverpa armigera* by spore-δ-endotoxin complex of different strains of *Bacillus thuringiensis* at 7 days exposure was evaluated through long term bioassays. Comparative studies in the development of test insect at 7 days exposure to the toxins of HD-I-S-1980 (16000 IU/mg) vs HD-244 (24,000 IU/mg) indicated non-significant differences in survivors at different developmental stages. However, both the toxins caused delayed effect on larval period. The changes in larval period were directly proportional to the log of concentration of the spore-crystal complex in the diet. The influence of toxin concentration had inverse correlation with the rate of pupation and adult emergence.

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

Introduction

Helicoverpa armigera (Huebn.) is of particular economic importance as a pest of many crops in Pakistan and it is also widely distributed on many host plants throughout the tropics and subtropics. Furthermore, *Heliothis* species have always been a global problem. There is no doubt that *Helicoverpa* spp. increased in importance largely because of chemical pesticides. The magnitude of *Heliothis* problem can be seen by the fact that nearly 30% of insecticides produced in the world are used to control different species of *Heliothis* (Ahmad, 1991).

The ability of insects to overcome and adapt to stresses of their environment has resulted in developing resistance to chemical insecticides. Insect resistance to chemical insecticides is well documented (Georghiou and Lagunes, 1988; Srivastava, 1995). *Bacillus thuringiensis* (Berliner) is a bacterium that produces toxic protein crystals during sporulation. The specific toxicity of these crystals against pest insects provide the basis for the use of this organism as biological insecticides (Adang, 1991). One cannot ignore the fact that preparation of *B.t.* based microbial pesticides have made inroads into the international commercial pesticide market. The role of *B.t.* based bio-pesticides or chemical pesticides in insect control mainly deals with the immediate effect of pathogens or chemicals on treated individuals but an ultimate goal of insect control is to save the crop, time to death is immaterial if no more or slow feeding takes place.

Burges (1981) stated that beyond the mortality caused by microbial insecticides, sub-lethally infected survivors are debilitated, delaying population recovery. Smirnoff further stated that population surviving chemical treatment were more vigorous than control population or the survivors of *Bacillus thuringiensis* applications.

This article evaluates the fate of the larvae survived after seven days exposure to spore-crystal complex of *Bacillus thuringiensis* strains. Comparative studies have been done on the development of *Helicoverpa armigera* larvae and their interaction with spore-δ-endotoxin complex of two strains HD-244 & HD-1-S-1980 having different potencies 24,000 IU/mg and 16,000 IU/mg respectively.

Materials and Methods

Long term bioassay procedure used to study the effect of spore-δ-endotoxin complex on the development of *H. armigera*: Rearing of the test insect, *H. armigera* was done on the artificial diet in laboratory, formula developed by Ahmed (1983) and neonate (1st instar) larvae were used in the bioassay. Two strains of *Bacillus thuringiensis* ssp. *kurstaki* (from the United States Department of Agriculture (USDA) namely HD-244 and reference standard strain HD-I-S-1980 having predetermined potency of 24,000 IU/mg (Khalique and Ahmed, 2001) and 16,000 IU/mg (arbitrary potency, Versoi, 1981) respectively.

Concentration No.	Preparations	Final conc. per ml after diet incorporation
1	Toxin + 100 ml BS*	60.0
2	45 ml HC + 15 ml BS*	45.0
3	25 ml HC + 25 ml BS*	30.0
4	25 ml # 2 + 25 ml BS*	22.5
5	25 ml # 3 + 25 ml BS*	15.0
6	25 ml # 4 + 25 ml BS*	11.25
7	25 ml # 5 + 25 ml BS*	07.5
8	25 ml # 6 + 25 ml BS*	05.625
9	25 ml # 7 + 25 ml BS*	03.75

Fig. 1: Diagrammatic presentation for the preparation of nine serial dilutions of spore-crystal complex of *Bacillus thuringiensis* (Berliner).

Nine serial dilutions (Fig. 1) of both the strains were prepared in buffered solution pH 7.0 (Khalique, 1995). 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 diet. The rest of the toxin concentration from 45.0, to 3.75 μg/ml diet were prepared from suspension number 2 to 9 respectively (Versoi, 1981) (Fig. 1).

After thorough mixing of 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 45 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 31 ± 3°C and 65 to 85% RH. Each experiment was replicated three times in different days and 25 larvae were used for each replicate.

The larvae (survivors) in all replicates of each concentration which escaped from death after seven days feeding of toxin were transferred to toxin-free or normal diet and allowed to develop till pupation was achieved. 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 for larval feeding.

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 hours of pupation, and placed on a blotting paper in a wide mouth glass jar for adult emergence. Mortality data at seven days, at pupation and at emergence were subjected to Probit analysis using a computer programme (Reymond, 1985) for working out LC_{50} and LC_{90} at 95% confidence limit.

Results

Comparative studies on the mortalities and retarded affect in the development of *H. armigera* after 7 days exposure to δ-endotoxin spore complex of HD-I-S-1980 and HD-244: Cumulative mortalities caused by HD-I-S-1980 among larvae to 7 days, to pupation and to emergence (Table 1) indicate that LC_{50} for the larvae was 8.45, 6.47 (the LC_{50} decreased by about 2.00 μg/ml) and 7.67 μg/ml diet (decreased by about 1.00 μg/ml as compared to 7 days LC_{50}) respectively. The mortalities observed in untreated (control) were 2.7, 4.1 and 21.6% for 7 days, pupation and emergence, respectively. Consequently the differences between LC_{50s} and LC_{90s} were too large than the LC_{50s} which indicated low interaction of insect at LC_{90s} at all the stages. LC_{50s} of HD-244 for 7 days, to pupation and to emergence were 5.49, 4.42 and 5.03 μg/ml, respectively. The activity of HD-244 toxin to different developmental stages of *H. armigera* was higher than HD-I-S-1980 (Table 2).

The data on toxicity of HD-244 based on mortalities at 7 days at pupation and at emergence were regressed. The regression equations were Probits $Y = 3.7+1.68 \log_{10} X$, $Y = 4.2 + 1.30 \log_{10} X$ and $Y = 4.1 + 1.33 \log_{10} X$ respectively. The coefficient of determination for the three regressions of the developmental stages were highly significant, however the differences between the regressions were not significant.

The activity of HD-I-S-1980 δ-endotoxin-spore complex was analyzed against the mortalities of *H. armigera* at various development stages the intercepts and slopes of regression equation for all stages (Probits $Y = 3.6+1.56 \log_{10} X$, $Y = 3.7 + 1.56 \log_{10} X$ and $Y = 3.6 + 1.60 \log_{10} X$ for 7 days, pupation and emergence respectively) did not differ significantly with each other. The coefficients of determination for all the stages were highly significant.

Biological effects of various concentrations of HD-I-S-1980 and HD-244 on *H. armigera*

Effect of toxin on larval development and pupation: The average larval period of larvae control was 15.76 ± 0.44 days. In comparison with control, the average larval periods at the lowest concentration (3.75 μg/ml) of HD-I-S-1980 and HD-244 were 18.27 ± 0.80 and 19.33 ± 1.01 days respectively (Table 3). Similarly, with the increase in toxin concentration there was a slight increase in the larval period. At the second highest concentration (45.00 μg/ml), the larval periods were 21.40 ± 3.85 and 21.57 ± 3.19 in HD-I-S-1980 and HD-244 respectively while in HD-244, no larva could pupate at 60.0 μg/ml diet concentration. These results also indicated larval retardation in almost all the treatments of both the strains. The highest pupation achieved in control was 96.0 % while the pupation of larvae treated with lowest concentration (3.75 μg/ml) of both the toxins was decreased (54.1 %) while at the highest concentration (60.0 μg/ml), only 6.7% pupation was achieved by HD-1-S-1980 (Table 3). These results showed inverse correlation between pupation and concentration of toxin.

Table 1: Toxicity of HD-I-S-1980 spore-δ-endotoxin determined at various stages of development of *H. armigera* at 7 days exposure

Survival period	LC_{50} (μg/ml diet)	95% CL Min.	Max.	LC_{50} (μg/ml diet)	95% CL Min.	Max.
For 7 days	8.45	6.49	10.50	57.65	40.57	94.14
To pupation	6.47	4.86	7.41	45.24	38.53	70.24
To Emergence	7.67	5.22	7.42	47.40	55.26	93.64

Table 2: Toxicity of HD-244 spore-δ-endotoxin determined at various stages of development of *H. armigera* at 7 days exposure

Survival period	LC_{50} (μg/ml diet)	95% CL Min.	Max.	LC_{50} (μg/ml diet)	95% CL Min.	Max.
For 7 days	5.49	3.75	7.38	41.92	29.16	68.41
To pupation	4.42	2.83	6.14	32.62	23.38	52.29
To Emergence	5.03	3.18	6.53	35.34	26.93	57.77

Table 3: Influence of spore-δ-endotoxin of HD-I-S 1980 and HD-244 on the larval development *Helicoverpa armigera* after seven days exposure

Level of toxin (μg/ml diet)	HD-I-S-1980 No. of survivors /total	Average* larval period (days) ± SD	Pupation (%)	HD-244 No. of survivors /total	Average * larval period (days) ± SD	Pupation (%)
0.00	71/74	15.8±0.44	96.0	71/74	15.8±0.44	96.0
3.75	40/74	18.3±0.80	54.1	40/74	19.3±1.01	54.1
5.625	41/75	18.1±0.80	54.6	30/72	19.2±0.02	41.7
7.5	36/75	19.1±0.92	48.0	22/76	20.5±1.92	28.9
11.25	27/74	19.3±1.24	36.5	21/75	20.0±1.45	28.0
15.0	22/75	18.8±1.07	29.3	16/75	19.8±1.67	21.3
22.5	13/75	19.2±1.78	17.3	15/75	20.1±1.50	20.0
30.0	13/75	19.4±1.08	17.3	7/75	21.6±3.19	9.3
45.0	5/75	21.4±3.85	6.7	7/75	21.6±3.19	9.3
60.0	5/75	20.4±4.57	6.7	-	-	0

*Including 95% confidence limit

Table 4: Influence of spore-δ-endotoxin of HD-I-S 1980 and HD-244 on the pupal weight of *Helicoverpa armigera* after seven days exposure

	HD-I-S-1980		HD-244	
Level of toxin (μg/ml diet)	No. of survivors /total	Average*pupal weight (g) ± SD	No. of survivors /total	Average * pupal weight (g) ± SD
0.00	69/74	0.351±0.011	64/74	0.351±0.011
3.75	39/74	0.365±0.017	39/75	0.370±0.017
5.625	41/75	0.380±0.004	29/72	0.369±0.016
7.5	36/75	0.375±0.019	21/76	0.369±0.016
11.25	27/74	0.362±0.023	21/75	0.368±0.028
15.0	21/75	0.362±0.025	13/75	0.348±0.032
22.5	13/75	0.368±0.020	15/75	0.372±0.091
30.0	13/75	0.364±0.035	7/75	0.372±0.023
45.0	5/75	0.357±0.050	7/75	0.389±0.068
60.0	5/75	0.359±0.40	-	0.355±0.096

*Including 95% confidence limit

Table 5: Influence of spore- 8- endotoxin of HD-I-S 1980 and HD-244 on the pupal duration of *Helicoverpa armigera* after seven days exposure

	HD-1-S-1980			HD-244		
Level of toxin (μg/ml diet)	No. of survivors /total	Average* pupal period (days) ± SD	Emergence (%)	No. of survivors /total	Average * pupal period (days) ± SD	Emergence (%)
.00	58/74	10.97±0.23	78.4	58/74	10.97±0.23	78.4
3.75	36/74	11.39±0.96	48.6	35/75	11.43±0.41	46.7
5.625	36/74	11.92±0.70	48.6	26/72	11.92±1.03	36.1
7.50	33/75	12.00±0.63	44.0	18/76	11.61±0.64	23.7
11.25	22/74	11.68±0.49	29.7	19/75	11.11±0.75	25.3
15.00	18/75	11.33±0.84	24.0	15/75	10.67±0.87	20.0
22.50	12/75	11.00±0.86	16.0	13/75	11.08±0.82	17.3
30.0	11/75	11.27±0.86	14.7	7/75	11.14±1.18	9.3
45.0	5/75	11.0±2.15	6.7	6/75	10.67±1.21	8.0
60.0	5/75	11.0±3.72	6.7	0	--	--

* Including 95% confidence limit

Table 6: Time of death of *H. armigera* larvae after seven days exposure to spore-crystal complex of HD- 1-S-1980 and HD 244

	HD-I-S-1980					HD-244				
	Death (%) occurring during indicated period (days)					Death (%) occurring during indicated period (days)				
Level of toxin (μg/ml diet)	No. of dying /total	0-7	8-14	15-21	22-28	No. of dying /total	0-7	8-14	15-21	22-28
0.0	3/75	66.7	0.0	33.3	0.0	3/74	66.7	0.0	33.3	0.0
3.75	18/75	77.8	5.6	11.1	5.6	35/75	97.1	2.9	0.0	0.0
5.625	28/75	89.3	7.1	3.6	0.0	42/72	81.0	19.0	0.0	0.0
7.50	25/75	96.0	0.0	4.0	0.0	54/76	92.6	1.9	1.8	3.7
11.25	31/75	93.5	0.0	6.5	0.0	54/75	96.3	1.9	0.0	1.8
15.0	39/74	84.6	12.8	2.6	0.0	59/75	89.8	5.1	3.4	1.7
22.5	53/75	100.0	0.0	0.0	0.0	60/75	93.3	5.0	0.0	1.7
30.0	61/74	96.7	3.3	0.0	0.0	68/75	94.1	2.9	1.5	1.5
45.0	65/75	93.8	1.5	4.6	0.0	69/76	100.0	0.0	0.0	0.0
60.0	71/74	98.6	0.0	1.4	0.0	75/75	98.7	0.0	1.3	0.0

Effect of toxin on pupal weight and pupal period: Both the toxins did not cause any retardation (significant increase or decrease) in pupal weight (Table 4). The pupal weight of control pupae was a little less than the weight of the pupae raised from larvae treated with various concentrations of both the toxins. Larvae surviving toxin treatments may be more healthy and vigorous than the control larvae. Pupal period remained un-affected at all concentrations of the toxins (Table 5).

Effect of toxin on survival time: The time of death of larvae (Table 6) exposed to various concentrations of HD-I-S-1980 and HD-244. The highest mortalities in untreated and treated groups were recorded in the 1st week of exposure. The sudden drop in mortality occurred in the second week when survivors transferred to toxin free diet. The pattern of death rate was similar in both the toxins.

Discussion

The pattern of cumulative mortality response to HD-I-S-1980 and HD-244 for seven days exposure at all the stages of insect development was found similar and a similar response was observed in case of LC_{90s}. The regression lines of HD-1-S-1980 and HD-244 illustrated that cumulative response of HD-244 at 7 days was a little higher than HD-1-S-1980 indicating fast interaction of insect with the toxin.

It is also noted from the data that maximum mortality occurred during the first 7 days. Dulmage *et al.* (1978) ascertained that if the larvae dying during exposure are not taken into account and the LC_{50} values are calculated only from those larvae that survive exposure, than the LC_{50} values would be markedly higher, which is an indication of a considerable ability to recover from the toxin. The effect of *B. thuringiensis* exposure for a certain period against any insect is judged by a single criterion: death; (Dulmage, 1981). In the bioassays of the δ-endotoxin of *B. thuringiensis*, we measure only the deaths that occur within a specified interval of time (7 days) (Dulmage and Martinez, 1973). The potency of *B. thuringiensis* strains indicates its degree of susceptibility against a certain insect. In this study, *H. armigera* was found more susceptible to HD-244 (24,000 IU/mg) than HD-I-S-1980 (16,000 IU/mg). A comparative long term effect of both the toxins was traced. Such type of comparative studies on retarded effect of larvae surviving up to pupation and emergence (with respect to potencies) were reported by different investigators. Khalique *et al.* (1982a) studied the development of *H. armigera* on spore-δ-endotoxin complex of *B. thuringiensis* var. pakistani 145 and compared with HD-I-S-1971. Trottier *et al.* (1988) compared the weight gain by the survivors of the reference standard strain treatments and more toxic strains than the standard.

The study indicated that larval development period of *H. armigera* by the feeding of HD-244 and HD-1-S-1980 toxins (for 7 days) at different concentrations increased three to seven days than control. Present results supported the observation of Khalique and Ahmed (2001) that the increase in larval period of *H. armigera* was directly related to concentration of *B. thuringiensis* (HD-694) toxins present in the diet. Ajanta *et al.* (1999) also recorded that all concentrations of Biolap, Dipel and Biobit tested had an adverse effect on growth and development of *H. armigera* i.e. increase in larval period. Lenin *et al.* (2001) reported that proteins encoded by the newly cloned Cry2Aa gene (at the concentration of 2.3μg/μl) showed 71.4 % motality of *H. armigera* on the seventh day and all the survivors that escaped from Cry2Aa toxicity showed sever (81-99%) inhibition in larval growth. Gujar *et al.* (2000) reported that larval growth was considerably reduced in treatments with HD-1 Biolap and Dipel R formulations.

Martinez *et al.* (1999) reported that toxin feeding tests with Cry1Ac and Cry1Ab indicated that the toxins retarded growth and inhibited food intake of susceptible larvae, but did not significantly affect the larvae of resistant strain, since the histopathological damage was similar in both strains, it appears that resistant larvae could repair more readily the damage cells.

Murugan *et al.* (1998) reported that *Bacillus thuringiensis* ssp. *kurstaki* at different concentrations considerably inhibited the food consumption of *Helicoverpa armigera*. Approximate digestibility was decreased at higher concentrations of *B.t. kurstaki*. The efficiency of conversion of ingested and digested food was significantly reduced and the profiles of digestive enzymes were decreased by *Bacillus thuringiensis* ssp. *kurstaki* treatments.

The pupal weights of treated group HD-244 and HD-I-S-1980 did not show any significant difference or adverse effect irrespective of the difference in potencies. Khalique *et al.* (1982) reported that average weight of the pupae treated with 10.0 & 20.0 μg concentrations of HD-I-S-1971 attained less weight than *B. thuringiensis* 145. Trottier *et al.* (1988) reported that survivors of 11 superior strains (more toxic than standard) gained significantly less weight than did survivors of the reference standard treatments and this effect was most pronounced for the most toxic strain.

The average pupal duration of HD-I-S-1980 treated group did not differ from the average pupal duration of HD-244 treated group. Consequently, present results indicated that HD-I-S-1980 & HD-244 caused no retardation in the pupal period and pupal weight of *H. armigera*, after 7 days toxin exposure and subsequent transfer of survivors to untreated diet. Thus, present results correspond to Fast and Regnier (1984) that larvae which are transferred to diet without *B. thuringiensis* before irreversible damage could occur should feed and continue their development normally. These results are also in agreement with the findings of Afify and Matter (1969) who observed no significant effect of larval treatment with *B. thuringiensis* on pupal duration of *A. kuehniella*. The percentage of the larvae that survived and succeeded to pupate was reduced 54.1 % for those larvae reared on diet containing 3.75 μg/ml for both the toxins as compared with control (96.0 %). Salama *et al.* (1981) & Soliman *et al.* (1970) also observed considerable reduction in rate of pupation in toxin treated larvae. Alford and Homes (1986) also noted that there were no effect on the pupal weights of spruce budworm of any *B.t.* treatments.

It appears that the exposure of newly hatched larvae of *H. armigera* to low concentrations of spore-crystal complex of both strains (HD-1-S-1980 & HD-244) of *B. thuringiensis* for seven days led to mortality of the larvae, but survivors completed their life cycle with prolongation in generation period, this is mainly due to retardation of larval development. The retardation in larval development increased with the increase in their toxin concentrations.

References

Adang, M.J., 1991. *Bacillus thuringiensis* insecticidal crystal proteins: gene structure, action and utilization. Biotechnology for biological control of pests and vectors. Karl (Ed.) by CRC Press, Inc., pp: 3-24.

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.

Ahmad, M., 1991. *Heliothis*- A new challenge to Pakistan cotton. PAPA Bulletin. December, pp: 35-39.

Ahmed, K., 1983. Development of artificial diets for mass breeding of some lepidopterous insects. M. Phil thesis, Department of Zoology, University of Karachi.

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

Alford, A.R. and J.A. Holmes, 1986. Sub-lethal effects of carbaryl, aminocarb, fenitrothion and *Bacillus thuringiensis* on the development and fecundity of the spruce budworm (Lepidoptera:Tortricidae). J. Econ. Entomol., 79: 31-34.

Burges, H.D., 1981. Strategy for the microbial control of pests in 1980 and beyond.Microbial control of pests and plant diseases 1970-80. H.D. Burges (Ed.). Academic Press. New York, London, pp: 797-836.

Dulmage, H.T. and E. Martinez,. 1973. The effect of continuous exposure to low concentrations of the δ-endotoxin of *Bacillus thuringiensis* on the development of the tobacco budworm, *Heliothis virescens*. J. Invertebr. Pathol., 22: 14-22.

Dulmage, H.T. and C.C. Beegle, 1980. Cooperative programme for studies on the spectrum of activity of the δ-endotoxin produced by *Bacillus thuringiensis*. Standard Technique Report No. 1-80.

Dulmage, H.T., 1981. Insecticidal activity of isolates of *Bacillus thuringiensis* and their potential for pest control. Microbial control of insect pests and plant diseases 1970-80, H.D. Burges (Ed.). Academic Press, New York, London, pp: 193-222.

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) Canadian Entomol., 116: 123-130.

Georghiou, G.P. and A. Lagunes, 1988. The occurrence of resistance to pesticides: Cases of resistance Reported World Wide through 1988 FAO, Rome, pp: 325.

Gujar, G.T., K. Vinay, K. Archana, V. Kalia, A. Kumari, 2000. Bioactivity of *Bacillus thuringiensis* against the American bollworm, *Helicoverpa armigera* (Hubner) Ann. Pl. Prot. Sci., 8: 2, 125-131.

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., 1995. Studies on disorientation in the development of *Helicoverpa* (*Heliothis*) *armigera* (Hübn.). after exposure to spore-crystal complex of *Bacillus thuringiensis* Berliner. Ph.D. thesis, Department of Zoology Karachi University.

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

Lenin, K., M. Mariam and V. Udaysuriyan, 2001. Expression of a Cry2Aa gene in an acrystalliferous *Bacillus thuringiensis* strains and toxicity of Cry2Aa against *Helicoverpa armigera*. World J. Microbiol. Biotechnol., 17: 3, 273-278.

Martinez, R.A.C., F. Gould and J. Ferre, 1999. Histo-pathological effects and growth reduction in a susceptible and a resistant strain of *Heliothis virescencs* (Lepidoptera: Noctuidae) caused by sub-lethal doses of pure Cry1A crystal proteins from *Bacillus thuringiensis*. Biocontrol Sci. Technol., 9: 2, 239-246.

Murugan, K., R. Babu, A. Pandey (Ed.), C.R. Socol (Ed.), and V.K. Josh, 1998. Impact of certain plant products and *Bacillus thuringiensis* Berliner subspecies *kurstaki* on the growth and feeding physiology of *Helicoverpa armigera* Hubner (Insecta: Lepidoptera: Noctuidae). International Conference on frontiers in Biotechnology, Trivandrum, India. J. Scientific Ind. Res., 57: 757-765.

Reymond, M., 1985. Presentation d'un programme d'analyse log-probit pour micro-ordinateur. Cal. ORSTOM, Ser. Ent. Med et Parasitol. 22.

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 endotoxin 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 Entomol., 66: 399-403.

Smirnoff , W.A., 1978. Forestry Chron., 54: 309-312.

Srivastava, C.P. 1995. Insecticide resistance in *Helicoverpa armigera* in India. Resistant Pest Management, 7: 4-5.

Trottier, M.R., O.N. Morris and H.T. Dulmag, 1988. Susceptibility of the Bertha armyworm, *Mamestra configurata* Walker (Lepidoplera, Nocluidea) to 61 strains from 10 varieties of *Bacillus thuringiensis* Berliner. J. Invertebr. Pathol., 51: 242-249.

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.