Table 1 *Evolution of CO_2 at different inoculum levels by four seedling pathogens of sugarbeet*

Fungus	Inoculum	Incubation period (in days) 4	8	12	16	20	24	Total
S. rolfsii	Control	1.43	0.23	0.13	0.0	0.63	0.13	2.55
	1%	13.46	11.73	10.10	8.70	6.56	4.33	54.88
	5%	16.56	14.90	13.10	11.90	9.53	7.26	73.25
	10%	18.93	17.33	16.46	14.40	13.20	12.40	92.72
P. ultimum	Control	1.90	1.23	0.70	0.63	0.40	0.10	5.96
	1%	4.70	12.23	9.00	6.60	3.66	3.66	39.55
	5%	10.70	17.16	12.26	10.30	5.93	5.76	62.11
	10%	15.70	18.40	14.46	14.73	10.33	10.06	83.68
R. bataticola	Control	1.43	0.13	0.13	0.0	0.63	0.13	2.45
	1%	9.40	6.53	3.96	2.36	3.83	1.23	27.31
	5%	11.60	6.86	7.53	4.40	4.53	2.43	27.35
	10%	12.50	7.26	7.80	4.46	7.86	3.13	43.01
R. solani	Control	1.90	1.23	0.76	0.63	0.40	1.10	6.02
	1%	6.93	11.40	6.50	4.93	2.96	3.30	36.02
	5%	11.50	16.70	9.63	8.70	5.76	5.53	57.82
	10%	16.26	18.96	18.30	10.36	12.80	13.60	90.28

Statistical analysis: D—Days; T—treatment; Int = interaction
S. rolfsii C D (D) = 0.50, CD (T) = 0.40, C D (Int.) = 1.01; *P. ultimum* C D (D) = 1.13, CD (T) = 0.93, C D (Int.) = 2.27; *R. bataticola* C D (D) = 0.78, CD (T) = 0.64, C D (Int.) = 1.56; *R. solani* C D (D) = 0.59, CD (T) = 0.48, C D (Int.) = 1.18

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COMPARATIVE TOXICITY OF *BACILLUS THURINGIENSIS* SUBSPECIES TO *SPODOPTERA LITURA* (F.)

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SPODOPTERA LITURA(F) is presently considered as one of the resistant, polyphagous pest species of field crops of economic importance in India. *S. litura*, commonly known as the tobacco caterpillar, is also one of the major tobacco pests. These caterpillars feed on the foliage in the nursery and in fields and often cause serious crop damages. This pest also attacks banana, safflower, sweet potato, Marsilla, cabbage, cauliflower, *Sesbania grandiflora*, cotton, tomato, castor, groundnut and soybean[1].

It is generally a well known fact that a majority of the crystalliferous bacilli which encompass *Bacillus thuringiensis* subspecies are toxic to Lepidoptera. However, some of the workers in the field[2,3] have stated that the subspecies of the same serotype differ in their toxicity, while others[4,6] have attempted fractionation of the crystal protein (δ-endotoxin) of *B.thuringiensis* var *Kurstaki* (HD-1) subspecies and have shown that these fractions exhibit differential toxicities to Lepidoptera and Diptera.

Hence, it was of interest to test the pathogenicity of different subspecies of *B.thuringiensis* obtained from USA and our indegenously isolated subspecies, to *S. litura* (F), to obtaining an ideal entomopathogen to combat this pest species more effectively (table 1.).

Lyophilised *B.thuringiensis* subspecies cultures were received from Dr Clayton Beegle of Cotton Research Unit, Brownsville, Texas, USA. These were revived on nutrient agar medium and the pure clones were transferred on agar slopes.

The slope cultures of all the subspecies mentioned in table 1 were allowed to sporulate and then harvested in 10 ml aliquots of sterile distilled water, aseptically. These suspensions were then mixed well on a vortex mixer and heat shocked at 60°C for 30 min and 1% of each inoculum was added to 200 ml aliquots of sterile nutrient broth supplemented with 0.3% molasses in 500 ml capacity Erlenmyer flasks. These flasks were then transferred to EMENVEE shaker at 180 r.p.m. at room temperature until complete sporulation was obtained. The growth from all the flasks was checked microscopically for purity and sporulation. All the broth cultures were then centrifuged on a RC2 centrifuge at 7000 r.p.m. for 30 min. The spore-crystal mixtures thus obtained were standardized by agar plate-count method. Desired dilutions with concentrations of 10^8 and 10^9 spores/ml were prepared. Before using these suspensions in the bioassays, Tween-80 was added as an emulsifying agent at 1% level and all these were mixed thoroughly on a vortex mixer.

The bioassays were conducted at room temperature (28°C ± 1°C), using 1st-instar larvae of *S. litura* maintained in our laboratory. Small diet blocks about (5 × 5 mm) were dispensed in clean 100 ml pyrex beakers. One hundred microlitres (100 µl) of each suspension were added to the surface of the diet and allowed to get absorbed. Ten first-instar larvae were then added to each of the beakers which were then covered with fine muslin cloth pieces. Untreated control diet beakers were kept with equal number of larvae, under identical conditions. Mortality counts were recorded every 24 hr for 8 days. These experiments had 3 replicates per organism and each experiment was repeated five times. The results presented in tables 2 and 3 show that the subspecies HD-1, 137, 228, 229, 244, 283, 549 (ISPC-1) 709 (ISPC-4) and ISPC-7 belonging to subspecies *aizawai*, *kurstaki* and *kenyae* were effective both at 10^8 and 10^9 spores/ml dose levels. However, subspecies HD-2, 125, 133, 241 and 263 belonging to the subspecies *tolworthi* and *thuringiensis* have shown negligible toxicity to this insect larvae.

Relative toxicity of different subspecies of *B.thuringiensis* has been studied[7] and it was shown that serotype-7 (subspecies *aizawai*) was most toxic than the other subspecies tested namely *kurstaki*, *subtoxicus*, *tolworthi* and *darmstadiensis*. Low susceptibility of the same pest species was shown[8] by ten subspecies of *B.thuringiensis* including *galleriae*, *darmstadiensis*, *sotto*, *morrisoni* and *tolworthi*. Moderate toxicity of the subspecies *entomocidus* and *thuringiensis* to this same pest was also reported[9]. Whereas, *B.thuringiensis* var *entomocidus* was shown to have the highest potential activity among the 17 subspecies they had screened[8].

Our studies thus indicate that the subspecies *kenyae*, in addition to others namely *aizawai* and *kurstaki* are also effective against *S. litura*. Also it is evident from the data presented in table 3, that our subspecies *kenyae* (ISPC-1 = HD-549), isolated from *Ephestia cautella*, *kurstaki* (ISPC-4 = HD-709), isolated from *Culex fatigans* larvae and *kenyae* (ISPC-7) isolated from BerHairy caterpillar *Thiacidas postica* were toxic and gave quick mortalities of *S. litura* larvae after 72 hr, when compared with other subspecies tested, (table 3). Our subspecies ISPC-1 and ISPC-4 were toxic to both Lepidoptera and Diptera[11], while ISPC-7, though belonging to subspecies *Kenyae*, still was found to be pathogenic to Lepidoptera only. Our observations are also in conformity with earlier studies[2–6] wherein differential toxicity of the members of the same subspecies has been confirmed. It is thus apparent that the indigenously isolated *B. thuringiensis* subspecies will help in controlling this serious pest

Table 1 *Organisms used in our Tests*

Howard Dulmage numbers	Serotype	Biotype	Subspecies
HD-1	3a,3b	III_2	*kurstaki*
HD-2	1	I	*thuringiensis*
HD-125	9	IX	*tolworthi*
HD-133	7	VII	*aizawai*
HD-137	7	VII	*aizawai*
HD-228	7	VII	*aizawai*
HD-229	7	VII	*aizawai*
HD-241	3a,3b	III_2	*kurstaki*
HD-244	3a,3b	III_2	*kurstaki*
HD-263	3a,3b	III_2	*kurstaki*
HD-283	7	VII	*aizawai*
Indigenously isolated			
HD-549 (ISPC-1)	4a,4c	IV_2	*kenyae*
HD-709 (ISPC-4)	3a, 3b	III_2	*kurstaki*
Not given (ISPC-7)	4a,4c	IV_2	*kenyae*

Table 2 *Cumulative mortality* data with B.thuringiensis subspecies at 10^8 spores/ml dose using lst-instar larvae of S. litura*

HD-numbers	Days after treatment 2	3	4	5	6	7	8
1	36.66	36.66	36.66	36.66	53.32	53.32	73.32
137	–	56.66	59.99	59.99	63.32	63.32	69.98
228	–	23.33	23.33	29.99	29.99	29.99	39.99
229	83.33	83.33	83.33	83.33	83.33	93.33	93.33
244	–	53.33	53.33	53.33	73.33	73.33	86.66
283	–	70.00	70.00	70.00	70.00	70.00	76.66
549 (ISPC-1)	22.96	37.77	72.58	78.50	82.94	85.16	85.16
709 (ISPC-4)	40.76	67.42	93.34	96.30	96.30	96.30	96.30
Not given (ISPC-7)	20.00	43.70	61.47	71.84	72.58	74.80	74.80

* The results presented herein are an average of five individual experiments with 3 replicates/ experiment. All the mortalities were corrected by using Abbott's[10] formula, 1925.

Table 3 *Cumulative Mortality* data with B.thuringiensis subspecies at 10^9 spores/ml dose using 1st-instar larvae of Spodoptera litura.*

HD-number	Days after treatment 2	3	4	5	6	7	8
1	43.33	43.33	43.33	43.33	96.66	96.66	96.66
2	–	–	–	–	–	–	–
125	–	10.00	10.00	10.00	10.00	10.00	16 66
133	–	3.33	3.33	9.99	18.32	23.32	26.65
137	80.00	80.00	80.00	80.00	96.66	96.66	96.66
228	53.33	53.33	53.33	53.33	73.33	73.33	79.99
229	100.00	–	–	–	–	–	–
241	–	–	3.33	3.33	3.33	3.33	3.33
244	76.66	76.66	79.66	96.66	96.66	96.66	96.66
263	–	16.66	36.66	36.66	36.66	36.66	36.66
283	53.33	53.33	53.33	53.33	73.33	73.33	79.33
549 (ISPC-1)	33.66	99.99	–	–	–	–	–
709 (ISPC-4)	40.99	96.66	–	–	–	–	–
Not given (ISPC-7)	27.77	97.77	–	–	–	–	–

• The results presented herein are an average of five individual experiments with 3 replicates/experiment. All the mortalities were corrected by using Abbott's formula[10].

of various field crops, ornamental plants and pests of medical importance.

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TISSUE LACTIC ACID AND GLYCOGEN LEVEL OF MOLLUSCS EXPOSED TO Cu AND Hg

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OF late, there has been a growing awareness of the toxic effects of trace metals on aquatic animals[1-5]. Most of the studies refer to acute toxicity and uptake kinetics. The present communication is on the effect of two trace metals *viz* Cu and Hg on the lactic acid and glycogen levels in the case of two commercially important bivalve molluscs, *Perna viridis* (Linnaeus) and *Villorita cyprinoides* var *cochinensis* (Hanley).

The animals acclimatized in the laboratory in filtered sea water of habitat salinity and ambient temperature, were exposed to sublethal levels of the metal (tables 1 and 2). The experimental conditions were the same as described earlier[6]. Individual animals were killed at definite intervals of time and soft parts used for determining lactic acid. To estimate glycogen, muscle or liver from 3 to 4 animals were dissected out and pooled for further analysis. Glycogen and lactic acid were estimated by standard methods[7-8]. Results

Table 1 *Change in the tissue lactic acid levels on exposure to Cu/Hg*

Concentration of metal ions (ppm)	Lactic acid μg/g wet wt ± S.D. Exposure time 2 hr	4 hr	24 hr
Perna viridis (Salinity 25‰, temp. 28°C)			
Cu			
0.5	163.56 ± 15.39	190.78 ± 11.07	392.18 ± 33.43
1.0	204.12 ± 21.46	231.95 ± 21.02	445.74 ± 48.82
2.0	297.50 ± 26.08	327.44 ± 18.50	621 44 ± 58.06
Control	48.25 ± 4.13	43.80 ± 3.52	46.61 ± 3.76
Hg			
0.5	127.26 ± 9 67	142.17 ± 9.46	344.17 ± 26.38
1.0	160.52 ± 18.30	176.56 ± 19.28	439.84 ± 37.15
Control	48 25 ± 4.13	46.55 ± 3.82	50.04 ± 4.16
Villorita cyprinoides (Salinity 10‰, temp. 28°C)			
Cu			
0.5	50.22 ± 3.73	74.80 ± 4.84	289.60 ± 33.50
1.0	85.69 ± 5.10	116.78 ± 8.52	423.54 ± 48.05
Control	26.50 ± 1.62	28.82 ± 1.90	37.48 ± 1.77
Hg			
0.5	49.23 ± 3.95	68.12 ± 5.07	254.84 ± 19.78
1.0	73.85 ± 4.68	110.34 ± 7.11	372.65 ± 46.68
Control	25.40 ± 1.44	23.65 ± 1.38	24.73 ± 1.56