in the lake. Differences in $\delta^{18}O$ and $\delta^{2}H$ values may also be caused by variable glacial input into the lake. Similarly, the outflow lakes from Lake Priyadarshini are observed to be more depleted than Lake Priyadarshini, particularly in $\delta^{2}H$.

The data set presented here establishes the current range of values of ^{3}H, $\delta^{2}H$, and $\delta^{18}O$ in lake waters in the Schirmacher oasis region, east Antarctica. Low levels of tritium indicate no human impact and the variations in $\delta^{2}H$ and $\delta^{18}O$ are in close correspondence with the relative contribution of old glacial cover and fresh snow precipitation.

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Spatial and temporal variation in susceptibility of the American bollworm, *Helicoverpa armigera* (Hübner) to *Bacillus thuringiensis* var. *kurstaki* in India

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Studies were carried out to determine the baseline susceptibility of the American bollworm, *Helicoverpa armigera* to *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) HD-1 and HD-73 strains for the populations collected from different places in India. The populations from Delhi (field), Raichur (F_1 and F_2) and Bangalore were least susceptible to the toxicity of *B.t.k.* HD-1. On the contrary, the Hyderabad and Madurai populations were most susceptible. *B.t.k.* HD-1 caused neonate mortality of 37.4% at 10 ppm and 68.6% mortality at 100 ppm after 96 h of treatment. Mehna and Nagpur populations were least susceptible to *B.t.k.* HD-73 whereas Guntur, Bapatla, Hyderabad, Madurai and Vijayawada populations were most susceptible. HD-73 caused mortality of 62.3% at 100 ppm and 91.7% at 500 ppm after 96 h of treatment. Temporal variations of insect mortality showed that LC_{50} (96 h) of *B.t.k.* HD-1 increased from 9.47 ppm in October 1998 to 51.04 ppm in December 1998 for the insect population on pigeonpea in Delhi. The baseline susceptibility studies show that there is a possibility of tolerance (resistance) in some populations. These studies are discussed in relation to their importance *vis-à-vis* growing use of *B.t.k.* and future cultivation of *B.t.* transgenic crops in the country.

AMERICAN bollworm, *Helicoverpa armigera* (Hübner) is an important polyphagous pest of cotton and many other crops of agricultural importance all over the world. It causes US $300 million worth of damage in legumes alone every year in India[1]. The total annual damage including all agricultural crops could be nearly US $1 billion. Dai and Guo[2] reported cotton yield loss of more than US $1 billion in China due to *H. armigera* alone during 1992. The pest has become serious with regular outbreaks and has developed resistance to almost all conventional insecticides including synthetic pyrethroids[3]. Therefore, it is natural that integrated pest management tactics including specific resistance management tactics have been advocated to control the pest[4–6]. Some of the management tactics recommend the

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use of biocontrol agents including use of *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*)[7–9] along with other components.

B. thuringiensis is a soil-borne bacterium pathogenic to insect pests and safe to higher animals[10]. It produces parasporal crystal (Cry) proteins during sporulation, which determine specificity of insecticidal action. *B. thuringiensis* is registered in India and elsewhere especially for the control of insect pests. The bacterial insecticides constitute nearly 90% of the worldwide biopesticide market. Moreover, transgenic crops having *cry* genes that were introduced first in USA beginning with Monsanto®'s Bollgard® cotton in 1996 are now being grown on more than 28 million acres in many countries and the area under transgenic cotton is likely to increase dramatically from 2 million acres at present. An important question that needs a satisfactory answer in the near future is as to how the pest complex is likely to adapt to the increasing use of bacterial insecticides and cultivation of *B. thuringiensis* transgenic crops.

In India, efficacy of *B. thuringiensis* has been reported against *H. armigera* either alone[11,12], or in combination with other pesticides[13] under field conditions. In order to develop an effective integrated pest management programme, there is a need for a database of baseline susceptibility of different populations of *H. armigera* to *B.t.k.* for monitoring temporal and spatial baseline susceptibility changes, and development of insect resistance. This paper therefore reports the spatial and temporal baseline susceptibility of *H. armigera* to *B.t.k.*

HD-1 and HD-73 were the standard strains received as gift from Pasteur Institute, France and Bacillus Genetic Stock Center, Ohio State University, USA, respectively. They were cultured on nutrient broth at 37°C for 72 h, then the spore–crystal complex harvested by centrifugation, and further processed to make acetone powder[14]. The acetone powder was stored at 4°C in aliquots till use. The total protein content of acetone powder was 3.73% in HD-1 and 2.29% in HD-73. The endotoxin content was determined by densitometry of electropherogram of proteins of acetone powder and found to be 0.538% in HD-1 and 0.282% in HD-73.

American bollworm was mostly collected as larvae from the fields of agricultural crops from different locations, viz. Delhi, Nagpur (Maharashtra), Hyderabad, Vijayawada, Guntur and Bapatla (Andhra Pradesh), Madurai (Tamil Nadu), Mansa and Mehna (Punjab), Raichur and Bangalore (Karnataka) (Figure 1). The larvae were reared till pupation on a modified semi-synthetic diet[15]. Adults emerging from the pupae were given 10% honey solution throughout their egg-laying period. About 10 pairs of adults were kept in each jar. The eggs laid on a markin cloth were kept in a separate jar at 27°C moistened with water. Upon hatching the neonate larvae were considered as belonging to the F_1 generation. The neonates belonging to different generations were used for study depending upon their availability.

The stock solution of acetone powder of *B.t.k.* strain was prepared in water, and one-fifth amount of *B.t.k.* solution of known concentration was mixed with a semi-synthetic diet broth at about 40°C before setting it in petri plates. After setting a diet containing two different treatment concentrations, viz. 10 and 100 ppm for HD-1 strain and 100 and 500 ppm for HD-73 strain, diet pieces of about 2 cm^3 each were offered to a group of 10 neonate larvae in a plastic cup that formed a replicate. There were five replications for each treatment. Data on mortality of neonates were recorded every 24 h for each replication for 96 h of treatment. At the end of the 96 h period, insects from each treatment were individually weighed and growth inhibition was estimated by comparing larval growth in treatments with that of the control.

For studies on temporal-dependent susceptibility of larvae, pigeonpea (var. UPAS 120) crop in full bloom infested with American bollworm during 1998 at a farm in the Indian Agricultural Research Institute (IARI), New Delhi was chosen. Moths were directly trapped in plastic jars and brought to the laboratory for laying eggs. Bioassays for HD-1 and HD-73 were carried out with 5–6 different concentrations ranging from 1 to 100 or sometime 200 ppm. There were five replications of ten neonate insects each. The full bioassay made use of a minimum of 250 larvae including a control. The mortality was recorded for each replication every 24 h for

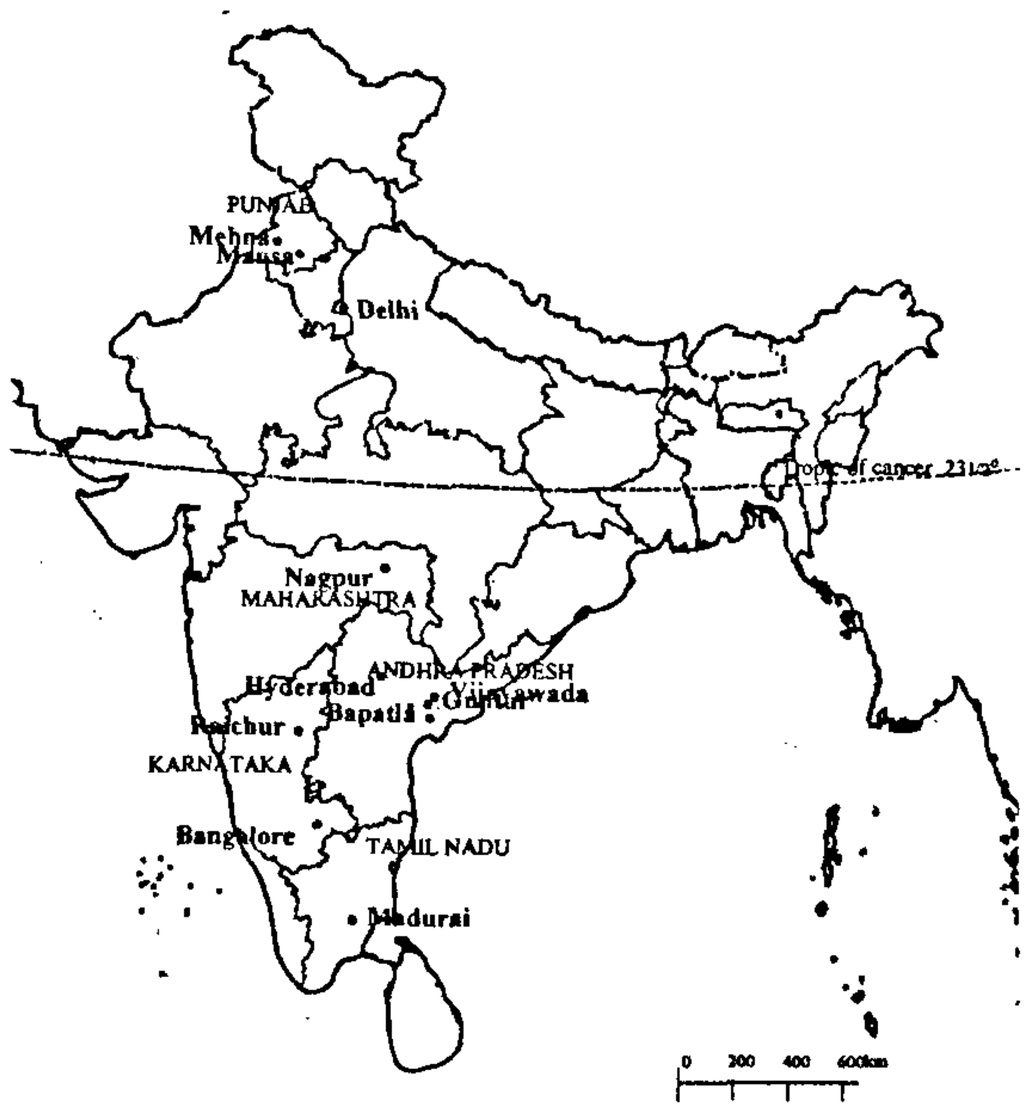

Figure 1. Geographic distribution of American bollworm populations in six states of India used in the present investigation.

96 h of treatment and pooled up for each treatment. Bioassays were carried out at 27 ± 2°C and 60–65% r.h. Data on mortality were subjected to probit analysis to estimate median lethal concentration (LC_{50})[16]. LC_{50} values at 96 h assay period were used for their consistency and sensitivity when compared to those at 48 and 72 h. Analysis of variance using least square design was carried out for comparing mortality and growth inhibition of different populations at each of the concentrations used at 5% level of significance according to the software programme of Indostat Services, Hyderabad, India.

The potency of HD-1 acetone powder against neonate larvae of *H. armigera* was studied by diet dip method; with LC_{50} (96 h) of 9.05 ppm (95% fiducial limits 1.97 and 17.68 ppm; slope 1.13). Similarly, LC_{50} (96 h) of HD-73 acetone powder against neonate larvae was found to be 6.21 ppm (95% fiducial limits 2.57 and 12.38 ppm; slope 0.66).

There were wide variations in the susceptibility of different populations of *H. armigera* to HD-1 (Table 1). Delhi (field), Raichur F_1 and F_2, and Guntur populations were the least susceptible ones (8.7–23.4%) at 10 ppm of HD-1. This was followed by the Bangalore population. Populations from Mansa, Mehna, Bapatla, Nagpur and Delhi (lab) were at par in their susceptibility and were in mid-range. Madurai and Hyderabad populations were most susceptible (70.4–80.8%) at 10 ppm concentration of HD-1. At 100 ppm concentration of HD-1, Delhi (field and lab), Bangalore, Raichur F_1 and F_2 and Mehna populations were least susceptible (39.5–59.7%) to HD-1. Populations from Mansa, Guntur, Bapatla and Nagpur were in the mid-range of susceptibility status. Vijayawada, Madurai and Hyderabad populations were most susceptible (85.8–93.3%).

When per cent mortalities at both concentrations of HD-1 strain were pooled together, Delhi (field), Bangalore, Raichur F_1 and F_2 were least susceptible (24.1–35.5%). This was followed by populations from Guntur, Mehna, Delhi (lab), Mansa, and Nagpur. Bapatla and Vijayawada populations showed mid-point response. The most susceptible populations (82.0–87.1%) were from Hyderabad and Madurai. HD-1 at 10 and

Table 1. Toxicity of *Bacillus thuringiensis* var. *kurstaki* HD-1 to neonates of the American bollworm *Helicoverpa armigera* (Hübner)

Population	Date of collection	Host crop	Date of assay	% Mortality			% Growth inhibition		
				10 ppm	100 ppm	Pool	10 ppm	100 ppm	Pool
Delhi	March 1998	Artificial	23 October	39.03	54.19	46.66	87.18	98.12	92.65
(lab)		diet	1998 (F_{10})	*38.63*	*47.40*	*43.01*	*69.02*	*82.12*	*75.57*
Delhi	14 September	Pigeonpea	5 November	8.72	39.52	24.12	75.00	96.73	85.86
(field)	1998	Cotton	1998 (F_2)	*17.18*	*38.96*	*28.07*	*59.99*	*79.58*	*69.75*
Raichur	25 September		23 October	15.33	54.98	35.16	83.01	86.23	84.62
(Karnataka)	1998		1998 (F_1)	*23.06*	*47.85*	*35.46*	*65.66*	*68.22*	*66.93*
Raichur	25 September	Artificial	1 December	23.40	43.88	33.64	90.42	98.11	94.27
(Karnataka)	1998	diet	1998 (F_2)	*28.93*	*41.49*	*35.20*	*71.97*	*82.10*	*77.00*
Hyderabad	20 November	Cotton	20 February	80.80	93.33	87.07	95.67	96.37	96.02
(Andhra Pradesh)	1998		1999 (F_3)	*64.04*	*78.05*	*68.93*	*77.99*	*79.02*	*78.51*
Bangalore	25 September	Artificial	22 October	25.60	40.48	33.04	65.48	94.73	80.11
(Karnataka)	1998	diet	1998 (F_1)	*30.40*	*39.50*	*34.95*	*54.01*	*76.73*	*65.35*
Mansa	4 November	Cotton	1 December	30.20	73.58	51.89	83.80	88.91	86.36
(Punjab)	1998		1998 (F_1)	*33.20*	*59.06*	*46.19*	*66.27*	*70.55*	*68.36*
Mehna	28 November	Cotton	18 January	30.90	59.72	45.31	97.26	98.39	97.83
(Punjab)	1998		1999 (F_2)	*33.78*	*50.60*	*42.20*	*80.68*	*82.71*	*81.70*
Guntur	27 October	Cotton	2 December	13.34	66.60	39.97	73.04	78.63	75.84
(Andhra Pradesh)	1998		1998 (F_1)	*21.42*	*54.70*	*38.06*	*58.72*	*62.47*	*60.60*
Bapatla	25 October	Cotton	4 December	47.98	83.05	65.52	78.57	83.50	81.04
(Andhra Pradesh)	1998		1998 (F_1)	*43.83*	*65.70*	*54.76*	*62.42*	*66.03*	*64.23*
Nagpur	22 February	Chenopodium	16 March	37.67	74.45	56.06	95.11	98.43	96.77
(Maharashtra)	1999		1999 (F_3)	*37.84*	*59.65*	*48.74*	*79.23*	*82.79*	*80.92*
Madurai	3 January	Lady's	1 April	70.35	93.73	82.04	94.88	97.87	96.38
(Tamil Nadu)	1999	finger	1999 (F_3)	*57.01*	*75.49*	*66.24*	*76.93*	*81.61*	*79.25*
Vijayawada	31 January	Tomato	20 April	54.01	85.84	69.93	93.96	98.17	96.07
(Andhra Pradesh)	1999		1999 (F_3)	*47.30*	*67.90*	*57.62*	*75.78*	*82.24*	*79.03*
SEm				4.419	4.411	4.487	1.326	0.885	1.083
CD				12.54	12.52	12.74	3.714	2.478	3.035

Figures in italics are arc sin values; analysis of variance was carried out with LSD at 5% level of significance. Growth inhibition is based on a mean of weights of 10 individual larvae at the end of 96 h treatment. Figures in parentheses are filial generations to which insects belonged at the time of bioassay.

100 ppm concentrations showed significant differences in toxicity causing mortality of 37.4 and 68.6%, respectively.

HD-1 at 10 ppm caused least growth reduction (65.5%) in the Bangalore population which was followed by Delhi (field), Guntur and Bapatla, and all three were on par. The most susceptible (95.1–97.3%) populations were Hyderabad, Nagpur and Mehna.

The concentration of 100 ppm of HD-1 caused least growth reduction (78.6%) in the Guntur population. This was followed by Raichur F_1 and Bapatla. Mansa and Raichur F_1 populations were at par. The populations from Bangalore and Hyderabad were in midorder of susceptibility range followed by Hyderabad and Delhi (field). The most susceptible (97.9–8.4%) populations were those collected from Nagpur, Madurai, Vijayawada, Delhi (lab), Raichur F_2 and Mehna. On pooling together growth reduction data for both concentrations of HD-1, the least susceptible (75.8%) population was that of Guntur. This was followed by Bangalore and Bapatla. Populations from Raichur F_1, Mansa, Delhi (field), Delhi (lab) and Raichur F_2 were in midorder of range of growth reduction. Raichur F_2 was at par with Hyderabad, Madurai and Vijayawada. The most susceptible (96.8–97.8%) populations were from Mehna and Nagpur. Both concentrations of HD-1 showed significant differences with mean growth reduction of 87.3% at 10 ppm and 94.7% at 100 ppm.

The baseline susceptibility response of different populations to *B.t.k.* HD-73 is presented in Table 2. Nagpur and Mehna populations showed least susceptibility (30.3–41.7%) to HD-73 at 100 ppm, followed by the Delhi population. The populations from Guntur, Madurai and Vijayawada were on par with Bapatla and in midrange of susceptibility. The most susceptible populations (78.5–82.6%) were from Hyderabad and Bapatla and were at par. The populations from Mehna, Nagpur and Delhi showed lowest larval mortality (73.1–87.3%) at 500 ppm of HD-73 while those Guntur, Bapatla, Hyderabad, Madurai and Vijayawada showed highest larval mortality (94.7–97.8%) at 500 ppm of HD-73. On pooling together mortality of different populations at two different concentrations, statistical analysis showed that HD-73 caused least mortality (54.0–57.4%) in Nagpur and Mehna populations. This was followed by Delhi (field). Guntur, Bapatla, Hyderabad, Vijayawada and Madurai populations showed highest larval mortality (82.7–88.7%) to HD-73 and were all at par. Both concentrations of HD-73 caused significantly different larval mortality, 62.3% at 100 ppm and 91.7% at 500 ppm.

As regards to growth reduction by *B.t.k.* HD-73, the concentration of 100 ppm HD-73 caused the least growth reduction (90.0–95.4%) in Delhi, Bapatla, Nagpur, Guntur, Madurai and Hyderabad populations that were at par. Vijayawada and Mehna populations showed highest growth reduction (95.9–97.1%) to HD-73 at 100 ppm, but were at par with all other populations except Delhi (field). HD-73 at 500 ppm showed variable growth reduction response in different populations. The population from Madurai showed minimum growth reduction (92.5%) while those from Mehna, Vijayawada,

Table 2. Toxicity of *Bacillus thuringiensis* var. *kurstaki* HD-73 to neonates of the American bollworm, *Helicoverpa armigera* (Hübner)

Population	Date of insect collection	Host crop	Date of assay (generation)	% Mortality			% Growth inhibition		
				100 ppm	500 ppm	Pool	100 ppm	500 ppm	Pool
Delhi	14 September	Pigeonpea	3 December	55.00	87.25	71.13	90.05	98.17	94.11
(field)	1998		1998 (F_3)	*47.87*	*69.25*	*58.56*	*71.61*	*82.22*	*76.95*
Mehna	28 November	Cotton	18 January	41.67	73.09	57.38	97.07	98.40	90.77
(Punjab)	1999		1999 (F_2)	*40.20*	*58.75*	*49.48*	*80.15*	*82.90*	*81.52*
Guntur	27 October	Cotton	27 January	68.05	97.78	82.92	95.40	95.75	95.58
(Andhra Pradesh)	1998		1999 (F_2)	*55.58*	*81.44*	*65.59*	*77.60*	*78.11*	*77.93*
Bapatla	25 October	Cotton	27 January	78.53	95.18	86.86	95.00	96.00	95.50
(Andhra Pradesh)	1998		1999 (F_3)	*62.40*	*77.32*	*69.86*	*77.06*	*78.47*	*77.76*
Hyderabad	20 November	Cotton	23 February	82.61	94.71	88.66	95.27	97.65	96.46
(Andhra Pradesh)	1998		1999 (F_3)	*65.41*	*76.70*	*71.06*	*77.44*	*81.17*	*79.27*
Nagpur	22 February	Chenopodium	20 April	30.27	77.75	54.01	93.27	97.22	95.25
(Maharashtra)	1999		1999 (F_3)	*33.38*	*61.86*	*47.62*	*74.96*	*80.40*	*77.83*
Madurai	3 January	Lady's	6 May	68.44	96.98	82.71	94.53	92.50	93.52
(Tamil Nadu)	1999	finger	1999 (F_4)	*55.82*	*79.99*	*65.43*	*76.47*	*74.10*	*75.27*
Vijayawada	31 January	Tomato	20 April	69.24	97.10	83.17	95.89	98.41	97.15
(Andhra Pradesh)	1999		1999 (F_3)	*56.32*	*80.20*	*65.78*	*78.30*	*82.75*	*80.52*
SEm				2.555	3.880	3.307	2.260	0.787	0.827
CD				7.361	11.17	9.524	6.372	2.220	2.331

Figures in italics are arc sin values; analysis of variance was carried out with LSD at 5% level of significance. Growth inhibition is based on a mean of weights of 10 individual larvae at the end of 96 h treatment. Figures in parentheses are filial generations to which insects belonged at the time of bioassay.

Delhi and Hyderabad showed maximum growth reduction (84.5–97.2%). On pooling together growth reduction data for both concentrations of HD-73, it was found that the Madurai population was least susceptible (93.5%) to the growth inhibitory effects of HD-73. This was followed by those from Guntur, Bapatla and Nagpur which were all at par. The populations from Delhi, Hyderabad and Vijayawada showed higher growth reduction to HD-73. The highest growth reduction (90.8–97.2%) was observed in populations from Vijayawada and Mehna. HD-73 caused growth inhibition of 95.3% and 97.1% at 100 and 500 ppm, respectively on pooling data of different populations.

The toxicity of HD-1 by diet dip was similar to that reported earlier[17,18]. Similarly, toxicity of HD-1 by diet incorporation method was comparable with that of Navon *et al.*[19]. Ingle *et al.*[20] also reported LC_{50} of 10 ppm for δ-endotoxin of *B.t.k.* against *H. armigera* for 24 h of bioassay duration. Chakrabarti *et al.*[21] reported LC_{50} of cry toxins against *H. armigera*. The differences in toxicity could be mainly attributed to strain, strain preparation, method of toxin delivery and duration of bioassay.

Temporal variations in the susceptibility of *H. armigera* collected from pigeonpea crop showed that the susceptibility of neonate larvae decreased with LC_{50} of 9.47 ppm from the time of monitoring in October 1998 to LC_{50} of 51.04 ppm in December 1998 (Table 3). The five-fold tolerance of *B.t.k.* by insects during late winter over that during early winter suggests a positive temperature relationship of *B.t.k.* toxicity like that of insecticides. This could be attributed to differential adaptation of insect growth and development to temperature.

The discriminating concentrations of 10 ppm close to LC_{50} of HD-1 and of 100 ppm close to LC_{90} were chosen for spatial baseline susceptibility studies. The populations from Delhi (field), Raichur and Bangalore were least susceptible to HD-1 while those from Madurai and Hyderabad were most susceptible to it. It is interesting to note that populations showing high adaptation to HD-1 were from the regions where use of *B.t.k.* and conventional insecticides was minimum. However, most susceptible populations belonged to regions from Andhra Pradesh and Tamil Nadu where a significant proportion of total pesticides was used.

In case of HD-73 too, populations showed similar susceptibilities like in HD-1. In Nagpur and Mehna pesticides were not extensively used. The most susceptible HD-73 populations belonged to Guntur, Bapatla, Hyderabad, Vijayawada and Madurai where pesticides were extensively used.

Thus it seems that conventional pesticides help in increasing susceptibility to *B.t.k.* The insect resistance to conventional insecticides does not extend to *B. thuringiensis* due to the latter's novel mode of action. Hence *B.t.k.* was found effective even against insecticide-resistant American bollworm[13,22]. In view of the fact that Andhra Pradesh accounts for nearly 33% of pesticide consumption, and 55% of pesticides are targeted towards cotton crop for the control of bollworms, it is natural to attribute higher susceptibility of insecticide-resistant populations from those locations to a non-conventional insecticide, *B.t.k.*, in the present investigation. Further, the variation in susceptibility of different populations could be attributed to the presence of *B.t.k.* tolerant genotypes and possible adaptation even to the limited use of *B.t.k.* in agroecosystem.

The variation in susceptibility of *H. armigera* populations from three different provinces in North China ranged widely, with LC_{50} for 2nd instar larvae varying from 63 to 114 ppm in 1995 and from 55 to 187.7 ppm in 1996. The higher tolerance of *B.t.k.* in 4 locations out of 6 studied was attributed to extensive use of *B.t.k.* in those regions[23]. Further studies by Shen *et al.*[24] showed development of resistance in *H. armigera* in five provinces in 1995. Shen *et al.*[25] determined baseline susceptibility and discriminating concentrations for *H. armigera* in China. Populations of *H. armigera* belonging to Yanggu (Shadong), Handan (Hebei), Xinxian (Henan), Xiaoxian (Anhui) and Fengxian (Jiangsu) showed resistance to *B.t.* when compared to the susceptible (SUS1) strain of *H. armigera*. Populations from Yanggu and Xinxian were also resistant to transgenic cotton. Resistance levels of host insects increased temporally in transgenic cotton. Similar studies in Punjab showed less susceptibility of *H. armigera* population from Ludhiana to Dipel 8L, a commercial *B.t.k.* formu-

Table 3. Temporal variation of susceptibility of American bollworm in pigeonpea at the IARI farm to *Bacillus thuringiensis* var. *kurstaki* HD-1

Date of insect collection	Date of bioassay	LC_{50} (96 h) ppm	Fiducial limits		Slope ± s.e.
			Lower	Higher	
3 October 1998#	7 October 1998	9.47	7.07	12.68	1.48 ± 0.16
20 October 1998#	24 October 1998	22.38	15.54	32.23	0.82 ± 0.12
31 October 1998#	5 November 1998	22.85	17.99	29.04	1.57 ± 0.15
23 November 1998*	13 December 1998	51.04	40.86	63.74	1.41 ± 0.16

Insects collected as moths# and larvae* from pigeonpea fields.

lation, when compared to Bhatinda[26] which is known for cotton cultivation and where use of conventional insecticides is also higher. The results of the studies on toxicity of HD-1 and HD-73 were similar to those of Salama *et al.*[27], Padidam[17] and MacIntosh *et al.*[28]. HD-73 produces only one kind of Cry toxin Cry1Ac while HD-1 produces five different Cry toxins, of which Cry 1Aa, 1Ab and 1Ac are the major ones.

Use of Cry1Ac toxin in transgenic crops, especially in cotton, makes it a more dominant toxin for crop protection and also one responsible for adaptation and development of insect resistance. In fact, more than 2 million acres of transgenic cotton having Cry1Ac toxin gene was cultivated in 1997. This acreage under transgenic cotton is increasing significantly in China and Australia. Further, transgenic crops like corn were cultivated on as large as 7 million acres in 1997. *Helicoverpa* spp., being polyphagous, are likely to adapt to the transgenics containing individual Cry toxins more easily and quickly as the transgenic area increases. The high dose toxin expression in the transgenic crop is considered a part of Monsanto®'s resistant management plan for bollgard® cotton. A high dose is defined as the one 25 times the amount needed to kill 99% of susceptible insects[29]. Hence, a high dose of 500 ppm of Cry1Ac producing HD-73 (which in the present investigation was close to LC_{90}, i.e. 544.98 ppm) was therefore used to evaluate tolerance of geographically different insect populations. Our investigations showed that besides adaptation to HD-1, some populations like Nagpur, Madurai and Mehna have shown more adaptability to HD-73. Similar investigations were carried out by Stone and Sim[30] who showed highly variable susceptibility in 15 geographically diverse populations of *Heliothis zea* and 12 geographically distinct populations of *Heliothis virescens* throughout southern USA to Cry1Ac. Wu *et al.*[31] reported geographic variation in susceptibility of *H. armigera* to Cry1Ac in China and found a wide range of susceptibility for mortality (100-fold) and a narrow range of growth inhibition (5-fold) in 23 geographic populations of the test insect. The results were similar to those of ours. Further studies showed likelihood of early and quick adaptation of *Helicoverpa* spp. to Cry1Ac, suggesting the possibility of development of resistance[29,32]. The present investigation suggests the possibility of development of tolerance (resistance) in *H. armigera* as use of *B.t.k.* as an insecticide increases and transgenic crops with Cry toxin genes are cultivated within the country. Extensive investigations on baseline susceptibility and regular monitoring for resistance could help in checking development of *B.t.k.* tolerance (resistance) and developing appropriate integrated pest management tactics.

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Resistance to 'CrylAc δ-endotoxin of *Bacillus thuringiensis*' in a laboratory selected strain of *Helicoverpa armigera* (Hubner)

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The cotton bollworm, *Helicoverpa armigera* (Hubner) was selected for resistance to CrylAc in the laboratory. In the first 4–5 regimens of selection, there was no apparent change in the susceptibility of *H. armigera* to CrylAc. However, initial indications of resistance were clear after the 6th selection regimen, and by the end of 10th generation resistance increased 76-folds as reflected by the LC_{50} values. Similarly, resistance factors with respect to the EC_{50} increased to 34-fold by end of the 10th generation. The slope, which was relatively steep at 1.8 in the first generation, declined to 0.68 by the end of the 11th generation, indicating an increase in the number of resistant heterozygous individuals in the final population. A laboratory strain that was maintained without any selection pressure for 10 generations did not exhibit any change in susceptibility to CrylAc toxin.

Bt transgenic cotton expressing CrylAc is on the anvil of commercial release in India. The main target of the transgenic technology is the cotton bollworm *Helicoverpa armigera* (Hubner), which has been causing crop losses in India, estimated at US$ 290–350 million annually[1]. *H. armigera* has reportedly developed resistance to almost all groups of insecticides that have so far been used for its management[2], thus causing difficulties in field control. Resistance to *Bt* toxins expressed in plants is likely to develop in herbivorous insects under the right conditions[3]. Once in regular field use, insect resistance to CrylAc as well, is eventually expected to develop in lepidopteran insects, as has been the case with most toxins including insecticides. Also, the use of a single gene may lead to rapid development of resistance in species, which are more susceptible or even moderately susceptible to the toxin, thus reducing the impact of the technology as an effective lepidopteran insect management tool. Resistance development in insects against *Bt* toxins has been reported from field populations as well as laboratory-selected insects. Resistance to *Bt* toxins, as high as 1640-fold over a susceptible strain was found in a localized population of the diamond back moth, *Plutella xylostella* (L.) from Hawaii[3]. Laboratory selection programmes have generated resistance in Indian meal moth, *Plodia interpunctella* (Hub.)[4], Colorado potato beetle[5] and up to 10,000-fold in the cotton bollworm *Heliothis virescens* (Fab.)[6–8]. The main purpose of this study was to examine the potential of *H. armigera* to develop resistance to CrylAc under intense selection pressure in the laboratory.

The CrylAc proteins were produced according to Albert *et al.*[9], from *Escherichia coli* strains containing hyper-expressing recombinant plasmid vectors pKK223-3. The toxin was quantified on SDS-PAGE densitometry and diluted as six to ten concentrations (ranging from 10 to 20,000-fold) in distilled water. Forty per cent of the protein extracted from the recombinant *E. coli* cultures was found to comprise CrylAc toxin. Different concentrations of the toxin solutions were mixed thoroughly into a semisynthetic diet[10] pre-cooled to 55°C, at a rate of 2.4 ml of the toxin solution per 24 ml diet, and each concentration dispensed into a single 12-well 'ICN-Linbro' insect culture tray. A composite laboratory culture of *H. armigera* was initiated from pupae obtained from various parts of India from field-collected larvae. One-day-old larvae were released at the rate of one per well at a total of twenty-four larvae per concentration in three replicates on the diet incorporated with toxins. Larvae were transferred into toxin incorporated fresh diet trays, once in two days. Mortality was recorded daily till the sixth day, after which weights of the surviving larvae were recorded. The surviving larvae were then transferred on to fresh diet sans the toxin. All assays were replicated two to three times and pooled data was subjected to analysis. The assays were performed in the laboratory at conditions of 27 ± 1°C and 70% relative humidity. Median Lethal Concentrations (LC_{50}) pre-

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