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Research Article

Relative toxicity of subspecies of *Bacillus thuringiensis* against lepidopterous insect pests of agricultural importance

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ABSTRACT: The relative toxicity of various subspecies of *Bacillus thuringiensis* (*Bt*) against important lepidopterous insect pests was determined. *Bt kurstaki* HD-1 was toxic against all the five insect pests tested; however, with low toxicity against *Spodoptera litura.* Larvae of *Helicoverpa armigera* and *Pieris brassicae* were five fold more susceptible to *Bt kurstaki* HD-73 that produces only Cry1Ac toxin. However, *Bt kurstaki* HD-73 was non-toxic against the larvae of *S. litura* and *Spilarctia obliqua*. *Bt aizawai* HD-137 was equally active against *S. litura* and *Sesamia inferens* but non toxic against *P. brassicae* and *S. obliqua*. On the other hand *Bt tolworthi* strain was highly toxic against *P. brassicae* and *S. obliqua*. *Bt japonensis* T23 001was non-toxic to all the insect pests tested. Larval growth of *H. armigera* was severely inhibitied at extremely low concentration of *Bt kurstaki* HD-73 (EC_{50} 0.07 ng/ml) and *Bt kurstaki* HD-1 (EC_{50} 0.16 ng/ml). The toxicity of indigenous *Bt* strains belonged to subspecies *tolworthi* and *galleriae* were also promising against *H. armigera.*

KEY WORDS: *Bacillus thuringiensis,* Strains, Toxicity, Insect pests

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INTRODUCTION

Bacillus thuringiensis (*Bt*) is a gram-positive endospore forming soil bacterium characterized by the presence of parasporal crystal proteins, which are toxic to insects. Many *Bt* strains with different host spectra have been identified and classified into different serotypes based on their flagellar antigens (Hofte and Whiteley, 1989). Insecticidal activity and host spectrum of a given *Bt* strain is determined by the kind of its crystal toxins. Normally a strain synthesizes one to five toxins with varying toxicity and expression level. Few subspecies of *Bt* are developed into commercial products and used against insect pests of agricultural and public health importance.

Currently, *Bt* based formulations are the most widely used biopesticide worldwide. The consumption of *Bt* biopesticide in India is 131 MT for the year 2009-10 (http://ppqs.gov.in/Ipmpest_main.html). The most commonly used *Bt* formulations in many agro eco system are derived from *Bt* subspecies *kurstaki* HD-1 strain which was originally isolated in sixties by Dulmage (1970). *Bt* sprays are in growing demand in recent years on vegetables and other high value crops where high level of safety and selectivity are desirable and resistance to synthetic insecticides is a problem.

However, continuous use of a single *Bt* strain will not provide sustainable control of insect pests. The development of field level resistance has already been reported for *Plutella xylostella* in many countries due to repeated use of *Bt* formulations based on single strain (Tabashnik, 1994; Mohan and Gujar, 2002). Hence, use or rotation of formulations based on different *Bt* strains should be encouraged to sustain the efficacy and durability of *Bt* biopesticides. The knowledge on specificity and relative toxicity of *Bt* strains against insect pests is essential for appropriate selection and use of specific *Bt* subspecies based formulations. *Bt* is a natural source of wide range of insecticidal toxins that are yet to be fully harnessed. The present investigation was aimed at understanding the relative toxicity of 10 reference *Bt* strains against important lepidopteran insect pests of crops because these strains possess one or more Cry toxins which are lepidopteran insect specific.

METHODOLOGY

Culturing and spore-crystal preparation of Bt strains

Sporulating cultures of *Bt* (Table 1) from Luria Bertani broth (Tryptne 10 gm, yeast extract 5 gm, NaCl 10 gm in 1000 ml water) grown at 30 °C for 72 h were centrifuged,

the pellets containing spore crystal mixtures were washed (10,000 rpm for 10 min at 4 °C) once in 0.5M sodium chloride and then twice in ice cold sterile water. Spore-crystal powder was prepared from the pellet by acetone-lactose co-precipitation method as described by Dulmage *et al*., (1970). The resultant spore-crystal powder was stored at 4°C for further use.

Crystal protein was solubilized by dissolving spore-crystal powder in solubilizing buffer (10 mM sodium carbonate, 10 mM dithiothreitol, pH 10.0) for 4 h at 37°C. The content was centrifuged at 10000 rpm for 10 min. The supernatant containing Cry toxin was estimated by Coomassie brilliant blue dye G 250 binding method (Bradford, 1976) using bovine serum albumin as standard. Spore crystal powder was subjected to serial dilution in sterile water and heated at 85°C for 15 min to kill all vegetative cells. Aliquots from appropriate dilutions were spread plated on LB agar plates with sufficient replications and incubated at 30°C for 24 h. Viable spores, producing colonies were counted and spore load was estimated per mg powder. The details of *Bt* strains, Cry protein content and spore load are presented in Table 1.

Table 1. Details of spore-crystal mixtures of *Bacillus thuringiensis* strains tested

Bt strains	Sub species	Source	Cry genes reported (Mohan and Gujar, 2001)	Cry protein content (%)	Spore count/mg
HD-1 (serotype 3a,3b, 3c)	*kurstaki*	BGSC, Colubus, USA	*cry1Aa, cry1Ab, cry1Ac, cry2Aa, cry2Ab*	1.60	3.3×10^{10}
HD-73 (serotype 3a,3b)	,,	,,	*cry1Ac*	1.42	1.1×1^{09}
HD-137 (serotype 7)	*aizawai*	,,	*cry1Aa, cry1Ab, cry1Ca, cry1Da*	2.23	2.8×10^{11}
HD-125 (serotype 9)	*tolworthi*	,,	*cry9Ca*	1.14	4.6×10^{10}
HD-8 *(serotype* 5a,5b)	*galleriae*	,,	*cry1Ab, cry1Ac, cryICb, cry1D*	2.05	1.3×10^{11}
T23 001 (serotype 23)	*japonensis*	,,	*cry9*	2.31	1.7×10^{11}
MTCC 8995 (serotype 23)	*tolworthi*	MTCC, Chandigarh, India	ND	1.82	1.0×10^{10}
MTCC 8996 *(serotype* 5a,5b)	*galleriae*	,,	ND	2.82	1.3×10^{10}
MTCC 8997 *(serotype* 5a,5b)	*galleriae*	,,	ND	2.33	1.1×10^{12}
MTCC 8998 *(serotype* 5a,5b)	*galleriae*	,,	ND	2.16	1.0×10^{11}

Test insects

The insect pests tested in this study were: Pink stem borer, *Sesamia inferens* Walker; tobacco caterpillar, *Spodoptera litura* (F.); cabbage butterfly, *Pieris brassicae* (L.); fruit borer, *Helicoverpa armigera* (Hübner) and the Bihar hairy caterpillar, *Spilosoma obliqua* (Walker). All the insects were collected as eggs/larvae from their host plants such as rice, cauliflower and braccoli field. Except *S. inferens* and *H. armigea*, all other insect larvae were maintained on natural diet (cauliflower leaves) in laboratory. Chickpea and green gram based semi synthetic artificial diets were respectively used to rear *H. armigera* and *S. inferens* larvae. Adult insects were provided with 10% honey solution fortified with vitamin E as food. Optimum rearing conditions (27±2°C temperature, 60-80% relative humidity and 14L:10D h photoperiod) were maintained in the laboratory throughout rearing and bioassay period.

Bioassays

Cauliflower leaf disc dip assay (Mohan and Gujar, 2001) was adopted to test *Bt* strains for their efficiency. However, for *H. armigera* and *S. inferens*, artificial diets were used to evaluate toxicity. Matured cauliflower leaves were washed in sterile distilled water containing 0.1% Triton X-100 and shade dried. Leaf disc of 5cm dia was prepared and dipped in various concentrations of toxin solutions. The leaf discs were shade dried vertically and offered to larvae. The procedure given by Navon (2000) was followed to test *H. armigera* in artificial diet. About 2% of the water in diet was replaced with toxin solutions and antibiotics were not added to preserve toxin and spore activities. The toxin was incorporated into the diet when the diet temperature was around 50°C. The diet was mixed well in magnetic stirrer and then poured into small plastic cups.

For each *Bt* strain, five to six concentrations were prepared and newly hatched (0-12 h old, prestarved) 1st instar larvae were released. Each concentration was replicated four times with 40 larvae each.

Larval mortality was observed after 72 h. But, larval mortality and growth inhibition (larval weight) for *H. armigera* were observed after 6 days due to their delayed response. Bioassays were repeated on different dates and the mortality was pooled to produce a single fitted dose-mortality analysis. Experiments showing more than 10% control mortality were discarded and repeated. Corrected

per cent mortality was calculated using Abbott's formula. The mortality and larval growth inhibition data were subjected to probit analysis for the calculation of LC_{50}, EC_{50}, slope and fiducial limits by MSTAT C computer program. Precision of bioassays were ensured by ^{2} heterogeneity and 95% confidence intervals (Navon, 2000). The relative toxicity of *Bt* strains were calculated using *Bt kurstaki* HD-1 as reference strain because of its broad host range and toxicity. Two populations were considered significant ($P = 0.01$) if their 95% fiducial limit values of a given toxin did not overlap.

RESULTS AND DISCUSSION

The susceptibility status of *P. brassicae* and *S. obliqua* were collated in Table 2. The cabbage butterfly, *P. brassicae*, an important pest of cole crops in hilly region, showed five times more susceptibility to *Bt tolworthi* and *Bt kurstaki* HD-73 (LC_{50} values 0.26 and 0.28 ppm respectively) as compared to *Bt kurstaki* HD-1. However, it was insensitive to *Bt aizawai* HD-137. *S. obliqua*, a polyphagous pest of many crops, was also found not susceptible to *Bt aizawai* HD-137 and *Bt kurstaki* HD-1. Contrarily, it was equally susceptible to *Bt kurstaki* HD-1 and *Bt tolworthi* HD-125 (LC_{50} values of 1.61 and 1.96 ppm respectively).

Table 2. Toxicity of *Bacillus thuringiensis* strains against *Pieris brassicae* and *Spilarctia obliqua*

Bt strain	No. of larvae used	LC_{50} (ppm) 72 h	Slope ± S.E.	Fiducial Limits		χ^2-Value	DF	Relative toxicity[b]
				Lower	Upper			
P. brassicae								
Bt kurstaki HD-1	240	1.40	1.09 ± 0.16	0.750	2.310	2.21	3	-
Bt kurstaki HD-73	240	0.28	1.76 ± 0.39	0.127	0.452	2.61	3	5.0
*Bt aizawa*i HD-137[a]	240	-	-	-	-	-	-	
Bt tolworthi HD-125	240	0.26	1.72 ± 0.35	0.132	0.398	0.92	3	5.4
Bt galleriae HD-8	240	2.11	0.99 ± 0.08	1.193	3.572	1.3	4	-1.5
Bt japonensis T23 001[a]	200	-	-	-	-	-	-	
Bt tolworthi (MTCC 8995)	240	7.9	1.58 ± 0.20	5.13	11.85	10.5	3	-5.6
Bt galleriae (MTCC 8996)	240	8.42	1.43 ± 0.2	5.11	13.06	6.5	3	-6.0
Bt galleriae (MTCC 8997)	240	2.54	0.97 ± 0.13	1.40	4.20	3.4	3	-1.8
Bt galleriae (MTCC 8998)	240	4.63	1.55 ± 0.23	2.77	7.10	0.68	3	-3.3
S. obliqua								
Bt kurstaki HD-1	240	1.61	1.61 ± 0.26	0.275	0.658	7.8	3	-
Bt kurstaki HD-73 [a]	240	-	-	-	-	-	-	-
Bt aizawai HD-137[a]	240	-	-	-	-	-	-	-
Bt tolworthi HD-125	280	1.96	1.60 ± 0.19	1.372	2.852	7.51	4	-1.4
Bt galleriae HD-8	240	1.39	0.99 ± 0.08	0.930	2.674	2.3	4	1.2
Bt japonensis T23 001[a]	200	-	-	-	-	-	-	-
Bt tolworthi (MTCC 8995)	210	5.10	1.11 ± 0.30	2.43	11.85	7.21	3	-3.6
Bt galleriae (MTCC 8996)	210	3.71	1.23 ± 0.27	7.38	13.06	2.21	3	-2.7
Bt galleriae (MTCC 8997)	240	2.82	0.80 ± 0.11	1.20	6.03	5.9	3	-2.0
Bt galleriae (MTCC 8998)	240	6.34	1.23 ± 0.20	3.43	14.21	1.31	3	-4.5

[a]No toxicity; [b]Fold toxicity as compared to LC_{50} of *Bt kurstaki* HD-1 strain for the given insect species (negative indicates lower toxicity), DF = Degrees of Freedom

Susceptibility status of three noctuid insect pests (*H. armigera, S. litura* and *S. inferens*) to *Bt* strains were unique (Table 3). The *S. litura* was relatively tolerant to all the *Bt* strains tested. However, it was totally insensitive to *Bt kurstaki* HD-73. On the basis of non overlapping fiducial limits, *Bt aizawai* HD-137 was significantly better (LC_{50} of 11.3 ppm) than *Bt kurstaki* HD-1 strain against *S. litura*. *Bt aizawai* HD-137 was equally active against *S. litura* and *Sesamia inferens* but non toxic against *P. brassicae* and *S. obliqua*. The fruit borer, *H. armigera* exhibited higher susceptibility towards *Bt kurstaki* HD-73 and HD-1 with LC_{50} values of 0.05 and 0.27 ppm respectively. *Bt aizawai* HD-137 and *Bt tolworthi* HD-125 strains were 41.3 and 62.4 times less toxic to *H. armigera* as compared to *Bt kurstaki* HD-1. In turn, *Bt kurstaki* HD-73 was 5.4 times more active than HD-1 strain. It was highly susceptible to *Bt kurstaki*

HD-73 (LC_{50} 2.54 ppm) than *Bt kurstaki* HD-1. In addition to mortality all *Bt* strains caused severe larval growth inhibition of *H. armigera* (Table 4). *Bt kurstaki* HD-73 caused maximum growth inhibition (EC_{50} 0.00007 ppm) followed by *Bt kurstaki* HD-1 (EC_{50} 0.00016 ppm). Growth inhibition capabilities for *Bt aizawai* HD-137 and *Bt tolworthi* HD-125 were 19.4 and 36.0 times lower as compared to *Bt kurstaki* HD-1. *Bt galleriae* HD-8 was significantly more toxic (LC_{50} 6.5 ppm) to the larvae of *S. litura* as compared to *Bt kurstaki* HD-1(LC_{50} 20.1 ppm) and equally toxic as *Bt kurstaki* HD-1 to other insect species tested. *Bt japonensis* strain T23 001was non-toxic to all the insect pests tested. The toxicity of four indigenous *Bt* strains belonged to subspecies *tolworthi* and *galleriae* obtained from IMTECH, Chandigarh were found highly toxic against the larvae of *H. armigera*.

Table 3. Toxicity of *Bacillus thuringiensis* strains against noctuid insect pests

Bt strain	No. of larvae used	LC_{50} (ppm) 72 h	Slope ± S.E	Fiducial Limits		χ^2 Value	DF	Relativie toxicity[b]
				Lower	Upper			
			H. armigera					
Bt kurstaki HD-1	240	0.27	0.57 ± 0.08	0.116	0.762	1.56	3	-
Bt kurstaki HD-73	240	0.05	0.78 ± 0.10	0.028	0.103	3.1	3	5.4
Bt aizawai HD-137	280	16.85	3.37 ± 0.62	12.22	22.57	4.53	4	-62.4
Bt tolworthi HD-125	280	11.16	2.94 ± 0.69	7.257	15.14	5.9	4	-41.3
Bt galleriae HD-8	240	0.33	0.73 ± 0.08	0.193	0.572	1.3	4	-1.2
Bt japonensis T23 001[a]	240	-	-	-	-	-	-	-
Bt tolworthi (MTCC 8995)	200	0.61	1.48 ± 0.15	0.438	0.858	5.1	3	-2.3
Bt galleriae (MTCC 8996)	200	0.39	0.88 ± 0.10	0.230	0.670	1.6	4	-1.4
Bt galleriae (MTCC 8997)	200	0.10	0.75 ± 0.08	0.056	0.157	12.0	4	2.7
Bt galleriae (MTCC 8998)	240	0.33	0.73 ± 0.08	0.190	0.570	1.3	4	-1.2
			S. inferens					
Bt kurstaki HD-1	240	2.64	1.37 ± 0.20	1.610	3.708	4.6	4	-
Bt kurstaki HD-73	240	2.74	1.16 ± 0.19	1.529	4.041	0.17	4	-1.0
Bt aizawai HD-137	240	13.4	1.12 ± 0.18	9.332	20.48	0.33	4	-5.1
Bt tolworthi HD-125	240	10.05	1.44 ± 0.20	7.485	13.61	5.8	4	-3.8
Bt galleriae HD-8	240	1.12	0.93 ± 0.11	0.63	2.520	3.1	4	2.4
Bt japonensis T23 001[a]	240	-	-	-	-	-	-	-
Bt tolworthi (MTCC 8995)	240	13.70	0.90 ± 0.15	7.04	27.64	1.3	3	-5.2
Bt galleriae (MTCC 8996)	240	12.54	1.11 ± 0.16	6.562	24.32	1.2	3	-4.7
Bt galleriae (MTCC 8997)	240	13.86	1.01 ± 0.14	6.63	29.72	2.0	3	-5.2
Bt galleriae (MTCC 8998)	240	18.10	1.14 ± 0.16	9.50	40.63	5.8	3	-6.9
			S. litura					
Bt kurstaki HD-1	240	20.1	1.37 ± 0.30	11.93	33.09	3.4	3	-
Bt kurstaki HD-73[a]	240	-	-	-	-	-	-	-
Bt aizawai HD-137	240	11.3	1.21 ± 0.17	7.01	10.27	3.7	3	1.8
Bt tolworthi HD-125	240	13.3	1.07 ± 0.19	7.79	25.04	4.2	3	1.5
Bt galleriae HD-8	240	6.5	1.83 ± 0.21	5.11	8.47	4.8	4	3.1
Bt japonensis T23 001[a]	240	-	-	-	-	-	-	-
Bt tolworthi (MTCC 8995)	200	19.8	2.60 ± 0.34	16.3	24.80	4.8	3	1.0
Bt galleriae (MTCC 8996)	200	36.0	4.0 ± 0.65	30.7	43.9	1.8	3	-1.8
Bt galleriae (MTCC 8997)	240	7.3	2.58 ± 0.38	5.70	8.95	19.3	4	2.8
Bt galleriae (MTCC 8998)	240	5.1	1.41 ± 0.20	3.70	6.98	3.3	4	3.9

[a]No toxicity; [b]Fold toxicity as compared to LC_{50} of *Bt kurstaki* HD-1 strain for the given insect species(negative indicates lower toxicity), DF = Degrees of Freedom.

Table 4. Larval growth inhibition of *H. armigera* by *Bacillus thuringiensis* strains

Bt strain	No. of larvae used	EC_{50} (ppm) 6th day	Slope±S.E.	Fiducial Limits		χ2-Value	DF	Relative growth inhibition[a]
				Lower	Upper			
Bt kurstaki HD-1	240	0.00016	1.04 ± 0.08	0.00012	0.00022	1.56	3	-
Bt kurstaki HD-73	240	0.00007	0.86 ± 0.07	0.000046	0.0001	5.7	3	2.3
Bt aizawai HD-137	200	0.0031	0.82 ± 0.09	0.0021	0.0045	1.49	2	-19.4
Bt tolworthi HD-125	200	0.0057	1.14 ± 0.10	0.0041	0.0078	2.3	2	-36.0
Bt galleriae HD-8	180	0.00008	1.2 + 0.08	0.00057	0.0001	6.7	3	2.0
Bt japonensis T23 001[b]	180	-	-	-	-	-	-	-
Bt tolworthi (MTCC 8995)	240	0.00006	1.0 + 0.07	0.000042	0.000075	6.7	4	2.7
Bt galleriae (MTCC 8996)	200	0.00001	1.3 + 0.11	0.000008	0.000014	11.5	3	16.0
Bt galleriae (MTCC 8997)	200	0.00002	1.27 + 0.10	0.000013	0.000023	2.2	3	8.0
Bt galleriae (MTCC 8998)	200	0.00002	0.91 + 0.02	0.000016	0.000032	12.5	3	8.0

[b]Fold growth inhibition as compared to EC_{50} of *Bt kurstaki* HD-1 strain (negative indicates lower inhibition), DF = Degrees of Freedom, b No toxicity.

The specific toxicity of crystal proteins in a *Bt* strain is the basis for its use against a particular insect pest. Besides the Cry toxins, *Bt* also produces Vegetative Insecticidal Proteins (VIP). The spores are also toxic, often synergizing the activity of the Cry proteins. Strains sharing the same cry genes exhibit different host specificity due to differences in express level of *cry* genes (Porcar *et al*., 2000). The other important factor which decides specificity and toxicity of a given *Bt* strain is the insect species and its geographical origin (Tabashnik, 1994; Mohan and Gujar, 2000; Gonzalez-Cabrera *et al*., 2001).

The cabbage butterfly, *P. brassicae* was totally insensitive to *Bt aizawai* HD-137. Contrary to this finding Lecadet and Martouret (1987) reported its susceptibility towards *Bt aizawai* HD-137. However, in the present investigation *P. brassicae* was found highly susceptible to *Bt kurstaki* HD-73. It is clear that HD-73 expresses *cry1Ac* gene, and its absence in *Bt aizawai* HD-137 render it non toxic to *P. brassicae*. Although *Bt* HD-1 produces five different Cry toxins including Cry1Ac, its toxicity is five fold lesser than *Bt kurstaki* HD-73 strain to *P. brassicae* probably due to poor expression of *cry1Ac* gene in *Bt kurstaki* HD-1. The hairy caterpillar, *S. obliqua* was found equally susceptible to *Bt kurstaki* HD-1 and *Bt tolworthi* HD-8 but insensitive to *Bt* HD-73 and *Bt aizawai* HD-137 strains. Probably the *cry* gene combinations and their differential expression pattern in *Bt kurstaki* HD-1 and *Bt aizawai* HD-137 led to the former toxic and the latter non toxic against *S. obliqua*. *Bt tolworthi* HD-125 is highly toxic to these two insects and can be an effective alternative for *Bt kurstaki* HD-1 in case of resistance development.

Among the noctuid insects tested, the tobacco caterpillar, *S. litura* was insensitive to *Bt* HD-73 and only moderately susceptible to other subspecies. On the other hand *H. armigera* was highly susceptible to *Bt kurstaki* HD-73 and HD-1. Ogiwara *et al*. (1992) reported that over digestion of *Bt* protoxin by midgut proteases led to poor sensitivity of *Spodoptera littoralis* to *Bt* strains. This might be one of the reasons that *S. litura* is poorly sensitive to many *Bt* strains. *H. armigera* and *S. litura* are polyphagous in nature and occur together in crop ecosystems like cotton, pulses and vegetables in India. Transgenic crops expressing *cry1Ac* gene may not be effective against *S. litura* and *S. obliqua*. The conflicting nature of insect susceptibility can be resolved by either *Bt* strains in formulations expressing desired combination of *cry* genes through genetic manipulations or deploying two toxin strategies in transgenic crops. Multi toxin or multi strain approach also delays development of resistance in insects.

The effectiveness of *Bt kurstaki* HD-1 based formulations was reported against *Spodoptera litura* and *Spilarctia obliqua* (Sharma, 2000). Pandey *et al*. (2009) reported that *Bt aizawai* HD-137 was more toxic (LC_{50} of 0.50ppm) than *Bt kurstaki* HD-1 (LC_{50} 1.12 ppm) to the 1st instar larvae of *S. litura*. *Bt aizawai* HD-137 produces Cry1C toxin (Porcar *et al*., 2000) which is highly toxic to the larvae of *S. litura*. Toxicity of various *Bt* strains and toxins against *H. armigera* has been adequately studied from India, China and Australia. All the studies established that *Bt kurstaki* HD-73 or its toxin Cry1Ac is highly toxic to *H. armigera* (Chakrabarti *et al*., 1998; Gujar *et al*., 2000; Liao *et al*., 2002). In the present study, both *H. armigera* and *S. litura* were found equally susceptible to *Bt* subspecies *tolworthi* HD-125. However, Liao *et al*., (2002) observed that *H. armigera* strain from Australia was poorly susceptible to Cry9Ca1 toxin produced by *Bt tolworthi*. Physicochemical conditions of gut lumen, kind of midgut proteases and their role in protoxin activation and the presence of midgut receptor for *Bt* toxins and their binding affinity are some of the host mediated factors that determines specificity and

toxicity of a *Bt* strain/toxin towards a given insect species (Oppert, 1999; Ferre and van Rie, 2002; Mohan and Gujar, 2003). In addition to mortality, all *Bt* strains showed prominent larval growth inhibition in *H. armigera*. Much lower doses were required to suppress larval growth than for achieving mortality. The LC_{50} and EC_{50} values indicated that this insect population being the highly susceptible one against *Bt* than the *H. armigera* occurring from any other region (Padidam, 1992; Wu *et al*., 1999; Jalali *et al*., 2004). The study revealed a complex pattern of insect susceptibility towards a given *Bt* strain. Apart from *Bt* strain, the origin of insect strain also influences toxicity. Hence, combining the toxins from two or more *Bt* strains by means of genetic manipulation will enable a single *Bt* product to manage all lepidopterous insect pests occurring on a crop plant. Further, screening of various *Bt* strains and toxins for their toxicity against insect species occurring in a particular agro ecological region or crop eco system is essential for optimal use of *Bt* in pest management programmes. Extensive screening may also lead to development of potent *Bt* strain into product. The advantages of using *Bt* product over transgenic is the presence of various pathogenic factors other than Cry toxins, that may synergies insecticidal activity and delays the development of resistance in insects.

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