



## Laboratory evaluation of native *Bacillus thuringiensis* isolates against second and third instar *Helicoverpa armigera* (Hubner) larvae

C. Lalitha, T. Muralikrishna, S. Sravani and K. Devaki

### ABSTRACT

Second instar *Helicoverpa armigera* larvae were treated with *B.t.* strains isolated from soil samples and recorded mortality in the range of 16.67% to 94.44%. Maximum mortality was observed in reference strain HD1 which was on par with the *B.t.* strain 281 isolated from North Telangana Zone. There was no mortality of larvae in the untreated control. *B.t.* isolates 122 and 22 recorded 83.33% mortality. Lowest mortality was recorded in *B.t.* strains 252 and 261 isolated from North Coastal Zone. 50% of *B.t.* strains recorded more than 50% mortality. Among six *B.t.* strains isolated from bacteria infected silkworms except *B.t.* strain 432, remaining other *B.t.* strains showed more than 50% mortality. Third instar larvae of *H. armigera* were treated with native *B.t.* strains isolated from soil samples collected in different zones of A.P. and mortality was recorded in the range of 5.56% (*B.t.* strain 261) to 83.33% (HD1). Highest mortality was recorded in *B.t.* strain HD1 which was on par with the *B.t.* strains 22 and *B.t.* strains 44, 87, 122. No larval mortality was observed in the control. 35% of the *B.t.* strains showed more than 50% mortality. Among six native *B.t.* strains isolated from bacteria infected silkworms, 50% mortality was recorded in *B.t.* strains 431, 434, 440 and 447. Forty *B.t.* strains (12, 22, 25, 44, 53, 58, 65, 71, 83, 87, 91, 95, 106, 122, 139, 153, 169, 175, 182, 193, 206, 224, 229, 242, 281, 291, 299, 307, 317, 341, 351, 372, 376, 408, 422, 424, 431, 434, 440 and 447) recorded more than 50% mortality against second and third instar *H. armigera*.

**Key words:** *Helicoverpa armigera*, laboratory evaluation, native *Bacillus thuringiensis* strains

### INTRODUCTION

*Helicoverpa armigera* Hubner is a polyphagous pest that attacks more than 182 host plants belonging to 47 botanical families in the Indian sub continent and it is now estimated to feed on more than 200 plant species (Pawar, 1998; Chelliah, 2011). The indiscriminate use of chemical pesticides is assumed to be a serious cause of concern to human health and environment safety. Biopesticides used for pest management are environmentally safe, selective, specific in their action and easily biodegradable. Apart from insect pathogenic viruses and fungi, bacterium *Bacillus thuringiensis* (*B.t.*) is only microbe which has been successfully exploited commercially for the management of insect pests. *B.t.* accounts for 95% of the world market of microbial biological control agents due to the twin advantages of safety to natural enemies, honeybees etc and its rapid action against target insect pests (Vimaladevi *et*

*al.*, 2001; Anitha *et al.*, 2011; Li, 2012). It is highly effective against several lepidopteran pests of economic importance.

### MATERIALS AND METHODS

#### Maintenance of *H. armigera* larvae

*H. armigera* culture was maintained in semisynthetic diet as reported by Nagarkatti and Satyaprakash (1974). Grown up larvae were collected from field and were reared on artificial diet in individual vials.

#### Collection of samples

Soil samples were collected at a depth of 10-15 cm in polythene bags from different agro-climatic zones of A.P. (Table 1) and stored at 4° C until processed. The sodium acetate selection method

(Travers *et al.*, 1987) was followed to isolate *B.t.* from soil samples. Identification of *B.t.* strains was done by Gram staining (Capuccino and Sherman, 1992) and crystal staining (Sharif and Alaeddinoglu, 1988).

### Bioassay

Native *B.t.* isolates along with reference strain HD1 were used for bioassay. One loop of the individual isolates were inoculated in 30mL MGM broth in a conical flask and kept in a shaker for 72h at 200rpm. Bengal gram seeds were soaked in MGM broth *B.t.* culture ( $3.2 \times 10^5$  C.F.U/1mL) containing 0.2% Triton-X-100 for overnight and kept on a blotting paper and fed. One seed was fed to a larva in a vial individually. Three replications were maintained for each isolate with six larvae per replication. Larval mortality was recorded after 48hrs.

## RESULTS AND DISCUSSION

The results showed wide variation (5.56-94.4%) in mortality among *B.t.* strains, when treated against second and third instar *H. armigera* (Table 2).

### *B.t.* strains isolated from soil samples

Maximum mortality was observed in reference strain HD1 which was on par with the *B.t.* strain 281 isolated from Northern Telangana Zone (Table 1). Lowest mortality was recorded in two *B.t.* strains 252 and 261 isolated from North Coastal Zone. Fifty per cent of *B.t.* strains (60/120) recorded more than 50% mortality. The mortality range was between 16.67 and 94.44 per cent. Third instar *H. armigera* larvae when treated with native *B.t.* strains resulting 5.56% to 83.33% (HD1) mortality.

**Table 1.** Number of samples collected from different zones of A.P.

S. No	Zone	Isolate number	Total samples	Gram staining positive isolates	Crystal staining positive	
					Isolates	Total
<b>I. Soil samples</b>						
1	Southern Zone	1-100	100	49	4, 8, 12, 15, 21, 22, 25, 29, 32, 36, 44, 49, 52, 53, 57, 58, 61, 65, 67, 68, 71, 76, 77, 83, 87, 91, 94, 95, 99.	29
2	Scarce Rainfall Zone	101-164	64	34	103, 106, 109, 111, 113, 118, 121, 122, 123, 126, 128, 132, 134, 136, 137, 139, 140, 148, 150, 153.	20
3	Krishna Zone	165-200	35	22	165, 168, 169, 171, 175, 179, 182, 185, 188, 190, 192, 193, 195, 197.	14
4	Godavari Zone	201-245	45	20	203, 206, 211, 217, 224, 229, 232, 233, 242.	9
5	North Coastal Zone	246-280	35	17	247, 252, 254, 257, 258, 261, 264, 265, 267, 268, 270.	11
6	Northern Telangana Zone	281-320	30	15	281, 285, 289, 291, 299, 307, 311, 317.	8
7	Southern Telangana Zone	321-355	35	19	323, 326, 327, 333, 336, 341, 347, 349, 351.	9
8	Central Telangana Zone	356-396	41	16	364, 371, 372, 375, 376.	5
9	High Altitude and Tribal Zone	397-429	33	18	403, 405, 408, 411, 416, 422, 424, 425, 426	9
<b>II. Bacteria infected silkworms from Palamaner division</b>		430-685	256 larvae	21	431, 432, 434, 440, 441, 447	6

**Table 2.** Laboratory evaluation of native *B.t.* isolates against second and third instar *H. armigera*

Treatment ( <i>B.t.</i> Isolate No.)	II instar	III instar	Treatment ( <i>B.t.</i> Isolate No.)	II instar	III instar
<b>A. Soil samples</b>			<b>III. Krishna Zone</b>		
<b>I. Southern Zone</b>			<b>IV. Godavari Zone</b>		
1 (4)	27.78	22.22	50 (165)	22.22	11.11
2 (8)	50.00	44.44	51 (168)	50.00	38.89
3 (12)	66.67	61.11	52 (169)	61.11	55.56
4 (15)	33.33	27.78	53 (171)	50.00	38.89
5 (21)	22.22	11.11	54 (175)	55.56	50.00
6 (22)	83.33	77.78	55 (179)	44.44	38.89
7 (25)	66.67	50.00	56 (182)	61.11	55.56
8 (29)	38.89	27.78	57 (185)	22.22	16.67
9 (32)	22.22	16.67	58 (188)	33.33	27.78
10 (36)	44.44	33.33	59 (190)	50.00	38.89
11 (44)	77.78	72.22	60 (192)	44.44	38.89
12 (49)	33.33	27.78	61 (193)	55.56	50.00
13 (52)	55.56	38.89	62 (195)	44.44	33.33
14 (53)	72.22	66.67	63 (197)	38.89	33.33
15 (57)	38.89	33.33	<b>V. North Coastal Zone</b>		
16 (58)	66.67	61.11	64 (203)	38.89	27.78
17 (61)	44.44	33.33	65 (206)	72.22	66.67
18 (65)	72.22	66.67	66 (211)	33.33	27.78
19 (67)	33.33	22.22	67 (217)	33.33	22.22
22 (76)	38.89	27.78	68 (224)	50.00	50.00
23 (77)	44.44	33.33	69 (229)	50.00	50.00
24 (83)	66.67	66.67	70 (232)	44.44	33.33
25 (87)	77.78	72.22	71 (233)	50.00	44.44
26 (91)	66.67	61.11	72 (242)	61.11	55.56
27 (94)	44.44	27.78	<b>VI. Northern Telangana Zone</b>		
28 (95)	72.22	66.67	73 (247)	38.89	27.78
29 (99)	27.78	16.67	74 (252)	16.67	11.11
<b>II. Scarce rainfall Zone</b>			75 (254)	50.00	44.44
30 (103)	44.44	33.33	76 (257)	33.33	22.22
31 (106)	66.67	61.11	77 (258)	22.22	16.67
32 (109)	38.89	27.78	78 (261)	16.67	5.56
33 (111)	50.00	44.44	79 (264)	50.00	44.44
34 (113)	22.22	22.22	80 (265)	33.33	22.22
35 (118)	44.44	33.33	81 (267)	22.22	11.11
36 (121)	50.00	38.89	82 (268)	27.78	16.67
37 (122)	83.33	72.22	83 (270)	55.56	38.89
38 (123)	44.44	27.78	<b>VII. Southern Telangana Zone</b>		
39 (126)	27.78	22.22	92(323)	38.89	22.22
40 (128)	50.00	44.44	93(326)	33.33	22.22
41 (132)	27.78	16.67	94(327)	38.89	22.22
42 (134)	55.56	38.89	95(333)	38.89	27.78
43 (136)	44.44	33.33	96(336)	50.00	44.44
44 (137)	44.44	33.33	97(341)	66.67	55.56
45 (139)	72.22	66.67	98(347)	33.33	22.22
46 (140)	33.33	27.78	99(349)	38.89	22.22
47 (148)	38.89	27.78	100(351)	50.00	50.00
48 (150)	22.22	16.67	<b>VIII. Central Telangana Zone</b>		
49 (153)	66.67	61.11	101(364)	44.44	38.89
			102(371)	27.78	22.22
			103(372)	61.11	50.00
			104(375)	50.00	44.44
			105(376)	50.00	50.00
			<b>IX. High Altitude and Tribal Zone</b>		
			106(403)	11.11	0.00
			107(405)	33.33	22.22
			108(408)	55.56	50.00
			109(411)	61.11	44.44
			110(416)	22.22	16.67
			111(422)	61.11	55.56
			112(424)	66.67	66.67
			113(425)	50.00	33.33
			114(426)	27.78	22.22
			<b>B. Bacteria infected Silkworms</b>		
			115(431)	55.56	50.00
			116(432)	33.33	27.78
			117(434)	55.56	50.00
			118(440)	55.56	50.00
			119(441)	55.56	44.44
			120(447)	50.00	50.00
			121(HD1)	94.44	83.33
			122(control)	0.00	0.00
			S.Em±	3.89	3.86
			CD(P=0.05)	10.84	10.71

Highest mortality was recorded in *B.t.* strain HD1 which was on par with the *B.t.* strains 22. No larval mortality was observed in the control. *ie.* 35% 42/120) of the *B.t.* strains showed more than 50% mortality (Table 1).

In southern zone highest mortality was recorded in *B.t.* strain 22 which was on par with the *B.t.* strains 44 and 87. Lowest mortality was recorded in *B.t.* strains 21 and 32. 51.72% of *B.t.* strains showed more than 50% mortality (Table 1). Where as in third instar maximum and minimum mortality in *B.t.* strain 22 and 21, respectively.

Among the twenty *B.t.* strains evaluated from scarce rainfall zone, maximum mortality of second and third instar *H. armigera* larvae was observed in *B.t.* strain 122 which was on par with the *B.t.* strain 139. Lowest mortality was observed in *B.t.* strains 132 and 150. Highest mortality of *H. armigera* was observed in *B.t.* strains 169 and 182 which was on par with the *B.t.* strains 175 and 193 collected from Krishna zone.

In Godavari zone, maximum mortality of second instar *H. armigera* was recorded in *B.t.* strain 206. Minimum mortality was recorded in *B.t.* strain 217 and 211. Maximum mortality was recorded with *B.t.* strain 206 when tested against third instar *H. armigera* followed by the *B.t.* strain 242 whereas, 224, 229 strains recorded 50% mortality. Remaining other *B.t.* isolates showed less than 50% mortality.

Maximum mortality of second instar *H. armigera* was recorded in *B.t.* strain 270 collected from North Coastal Zone, which was on par with the two other *B.t.* strains 254 and 264 recorded 50 % mortality. Moderately higher mortality of third instar *H. armigera* was recorded in *B.t.* strains 254 and 264.

Among the eight native *B.t.* strains collected from Northern Telangana Zone, evaluated against second instar *H. armigera* maximum mortality was observed in *B.t.* strain 281 compared to other *B.t.* strains, followed by *B.t.* strain 317. Minimum mortality was recorded in *B.t.* strain 289. Highest mortality of third instar *H. armigera* was recorded in *B.t.* strain 281 compared to all other remaining *B.t.* strains. Except *B.t.* strains 289 and 311, remaining *B.t.* strains showed 50% mortality.

Maximum mortality in second instar *H. armigera* was recorded with *B.t.* strain 341 of Southern Telangana Zone, followed by *B.t.* strains 336 and 351. Among nine native strains of *B.t.* evaluated, highest mortality of third instar *H. armigera* was recorded in *B.t.* strain 341 which was on par with the *B.t.* strain 351.

Maximum mortality was recorded in second instar *H. armigera* treated in *B.t.* strain 372, while other four *B.t.* strains recorded the mortality in the range of 27.78 to 50.0 per cent. These strains were collected from Central Telangana Zone. Moderately high mortality was recorded in third instar *H. armigera* treated with *B.t.* strain 376 when compared to other four strains.

At High Altitude and tribal Zone, maximum mortality was recorded in second instar *H. armigera* was recorded in 424 and it was followed by 411 and 422 which were on par with the *B.t.* strain 408. Among the nine native *B.t.* strains evaluated maximum mortality of third instar *H. armigera* was recorded in strain 424 was on par with 408 strain. The bioassay results of all 120 native *B.t.* strains against second instar larvae of *H. armigera* with mortality of 16.67 to 94.44% is comparable to Xavier *et al.* (2007) who have isolated 30 *B.t.* strains from grain samples and soil samples from sericulture environment and subjected to

preliminary larvicidal assays against second instar larvae of *Helicoverpa armigera* at 300µg/mL showed 40 to 100% mortality.

The highest mortality of third instar *H. armigera* was recorded in *B.t.* strain HD1 which was on par with the *B.t.* strains 22 and *B.t.* strains 44, 87, 122. Daniz and Kornosor (1987) also reported that the third instar larvae of *H. armigera* showed mortality rate of 88% after 3 days of application of tarmac (*B.t.* sub sp *thuringiensis*) 0.15 µl concentration. According to Xavier *et al.* (2007) thirty *B.t.* isolates from grain samples and soil samples were toxic against *H. armigera*. Two isolates namely BTRX24 and BTRX28 showed higher mortality compared to other isolates.

### ***B.t.* strains from bacteria infected silk worms**

Except *B.t.* strain 432 all other five *B.t.* strains showed more than 50% mortality in second instar *H. armigera* (Table 2). In case of third instar *H. armigera* the larval mortality was recorded to the extent of fifty per cent when treated with *B.t.* strains of 431, 434, 440 and 447.

Similar results were reported by Gujar *et al.* (2000; 2004) according to which, HD-1@ 100ppm and 500ppm resulted in 62.3% and 91.7% mortality after 96 h treatment. Similar results were reported by Praveen *et al.*, (2001). It was found that HD-1 showed the highest toxicity to the fifth day larvae followed by Biobit and Biolep.

In the present study maximum mortality was observed in the reference strain HD1 which was on par with the *B.t.* strain 281 isolated from North Telangana Zone. Kulkarni and Amonkar (1988) also recorded 99 per cent mortality in first instar *H. armigera* larvae after two days of treatment with *B.t.* strains. *B.t.* 431, 434, 440 and 447 strains (4/6) isolated from bacteria infected silkworms showed more than 50% mortality of *H. armigera*.

### **Categorization of *B.t.* strains according to their toxicity levels against second and third instar *H. armigera***

Maximum (66/120) of *B.t.* strains gave 26 to 50% mortality of second instar *H. armigera* larvae. Only

5.04% *B.t.* strains showed more than 75% mortality. However, maximum (62/120) *B.t.* strains showed 26 to 50% mortality of third instar *H. armigera*. Minimum 2.52% (3/120) *B.t.* strains showed more than 75% mortality. In the present study none of the *B.t.* strains recorded 100 per cent mortality. Similar results were also reported by Lakshminarayana and Sujatha (2005) who recorded that none of the tested strains resulted in 100% mortality at the highest concentration even up to 6 DAT. Kumar (2002) observed larval mortality up to 70 per cent at 48h and 100 per cent at 72 h against *H. armigera* at a crude protein concentration of 10µg/200µl. Birdar (2003) also observed the maximum mortality by D1 isolate, followed by P1and reference strain HD1(94.60%) in the lab studies. Ajanta *et al.* (1999) found that the larval mortality after 48 h of exposure ranged from 12.69 to 76.77 per cent and 20.68 to 74.56 per cent in Biobit, Biolep and Dipel treated food, respectively.

#### ACKNOWLEDGEMENTS

The authors are grateful to the Acharya N.G. Ranga Agricultural University, for providing facilities and promoting the fellowship.

#### REFERENCES

- Ajantha, C., Kaushik, N. C. and Gupta, G. P. 1999. Studies of *Bacillus thuringiensis* on growth and development of *Helicoverpa armigera* Hubner. *Annals of Plant Protection Sciences*, **7**: 154-158.
- Anitha, D., Kumar, N. S., Vijayan, D., Ajithkumar, K. and Gurusubramanian, G. 2011. Characterization of *Bacillus thuringiensis* isolates and their differential toxicity against *Helicoverpa armigera* populations. *Journal of Basic Microbiology*, **51**(1):107-114.
- Cappuccino, J. G. and Sherman, N. 1992. *Microscopy a laboratory manual*. Third edition. The Benjamin publishing company, Inc. Newyork, USA. 521 P.
- Chelliah, A., Gupta, G. P., Karuppiah, S. and Kumar, P. A. 2011. Chimeric  $\delta$ -endotoxins of *Bacillus thuringiensis* with increased activity against *Helicoverpa armigera*. *International Journal of Tropical Insect Science*, **31**: 59-68.
- Daniz, S. and Kornosor, S. 1987. Determination of effectiveness of *Bacillus thuringiensis* Berliner against larvae of corn ear worm *Helicoverpa armigera* (Hub.) damaging cotton. In: *Turkey Entomological Kongresi Bildirileri*, 13-16 PP.
- Gujar, G. T., Vinay, K., Archana, K., Kalia, V. and Kumari, A. 2000. Bioactivity of *Bacillus thuringiensis* against the *Helicoverpa armigera* (Hubner). *Annals of Plant Protection Sciences*, **8**:125-131.
- Gujar, T., Kalia, V, Kumari, A. and Prasad, T. V. 2004. Potentiation of insecticidal activity of *Bacillus thuringiensis* sub sp. *kurstaki* HD-1 by proteinase inhibitors in the American bollworm, *Helicoverpa armigera* (Hübner). *Indian Journal of Experimental Biology*, **42**(2): 157-163.
- Kulkarni, U. V. and Amonkar, S.V. 1988. Microbial control of *H. armigera* part II. Relative toxicity of spores and crystals of *Bacillus thuringiensis* varieties to *H. armigera* and their efficacy in field control. *Indian Journal of Experimental Biology*, **11**: 174-178.
- Kumar, P. 2002. Evaluation of native *Bacillus thuringiensis* isolates. *M. Sc. (Agriculture) Thesis*, University of Agricultural Sciences, Dharwad.
- Lakshminarayana, M. and Sujatha, M. 2005. Toxicity of *Bacillus thuringiensis* var *kurstaki* strains purified crystal proteins against *Spodoptera litura* on castor, *Ricinus communis* (L.). *Journal of Oilseeds Research*, **22**(2): 433-434.
- Li, H. and Bouwer, G. 2012. Toxicity of *Bacillus thuringiensis* Cry proteins to *Helicoverpa armigera* (Lepidoptera: Noctuidae) in South Africa. *Journal of Invertebrate Pathology*, **109**: 110-116.
- Nagarkatti, S. and Satyaprakash. 1974. Rearing of *Heliothes armigera* on artificial diet. In: *Technical bulletin of Common wealth institute for Biological Control*, **17**: 169-173.
- Pawar, R. C. S. 1998. *Helicoverpa* a national problem that needs a national policy and commitment for its management. *Pestology*, **22**: 51-57.
- Praveen, P. M., Dhandapani, N. and Kennedy, J. S. 2001. Efficacy of *Bacillus thuringiensis* sub sp *kurstaki* (Berliner) formulations for the management of tomato fruit borer, *Helicoverpa armigera* (Hubner). *Pestology*. **25**:58-61.

- Sharif, F. A. and Alaeddinoglu, N. G. 1988. A rapid and simple method for staining of the crystal protein of *Bacillus thuringiensis*. *Journal of Industrial Microbiology*, **3**: 227-229.
- Travers, R. S., Martin, P. A. W. and Reicneldorfer, C. F. 1987. Selective process for efficient isolation of soil *Bacillus* spp. *Applied and Environmental Microbiology*, **53**:1263-1265.
- Vimaladevi, P. S., Ravinder, T. and Jaidev, C. 2005. Barley based medium for the cost effective production of *Bacillus thuringiensis*. *World Journal of Microbiology and Biotechnology*, **21**: 173-178.
- Xavier, R., Nagarathinam, P., Gopalakrishnan, Murugan, V. and Jayaraman, K. 2007. Isolation of lepidopteran active native *Bacillus thuringiensis* strains through PCR panning. *Asia*

*pacific Journal of Molecular Biology and Biotechnology*, **15**(2): 61-67.

---

**C. Lalitha, T. Muralikrishna, S. Sravani and K. Devaki**

Department of Entomology, Regional Agricultural Research Station, Acharya N. G. Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India.

Phone: +91 8985700447;

E mail: devaki\_kayam@rediffmail.com

---

#### **Manuscript history**

Received : 09.11.2011

Revised : 20.02.2012

Accepted : 21.04.2012