

## Laboratory evaluation of native *Bacillus thuringiensis* isolates against *Spodoptera litura* (Fabricius)

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### ABSTRACT

One hundred fourteen native *Bacillus thuringiensis* (*Bt*) strains isolated from soil samples were evaluated against I and III instar larvae of *Spodoptera litura* (Fab.) during 2009-10. The laboratory bioassays revealed that I and III instar *S. litura* larvae treated with 114 native *Bt* isolates showed mortality in range of 10 to 93.33%. Highest mortality was recorded in HD1 reference strain (93.33% and 76.67% in I and III instar larvae, respectively) which was on par with the native *Bt* isolates, 375 (90% and 70% in I and III instar larvae, respectively) and 416 (86.67% in I instar *S. litura* larva) isolated from Central Telangana and High Altitude and Tribal Zones. Next best treatments were found to be *Bt* isolate Nos. 4, 15, 21, 111 and 206 which resulted in 80 per cent mortality. Lowest mortality (6.67 %) was recorded in *Bt* strain 137 isolated from Scarce Rainfall Zone. No mortality was recorded in untreated control with respect to I instar and in *Bt* strains 8, 137, 148, 229, 268, 408, 425 and untreated control with respect to III instar *S. litura* larvae. 50.8% of the *Bt* strains (61/120) tested against I instar *S. litura* recorded more than 50% mortality.

**KEY WORDS:** *Bacillus thuringiensis*, laboratory evaluation, native strains, *Spodoptera litura* I and III instars

### INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae) is a polyphagous pest with high mobility and reproductive capacity widely distributed throughout tropical and temperate Asia, Australia and Pacific islands (Mohammad Monobrullah and Uma Shankar, 2008). *Spodoptera litura* (Fab.) devastates a large host range of more than 120 host plants (Ramana *et al.*, 1988). Indiscriminate use of chemical insecticides to control this pest has resulted in resistance to chemical insecticides, resurgence and deleterious effects to environment and non target organisms. In recent years microbial insecticides have become a viable alternative to control lepidopteran pests particularly *S. litura*. One of the most

important insect pathogens in the world today is the bacteria *Bacillus thuringiensis* accounting for 1-2% of the global insecticide market (Lambert and Peferoen, 1992). Quantification of the toxicity by insect bioassays is the only way to assess the potency of a strain for pest control. Keeping the importance of *Bt* in view, native *Bt* strains isolated from different zones of A.P. was evaluated against *S. litura* under *in vitro* conditions.

### MATERIALS AND METHODS

The studies were carried out at Regional Agricultural Research Station, Tirupati, A. P., India during 2009-10. One hundred and fourteen native *Bt* strains isolated from different zones of A.P. were

used for bioassay along with reference strain *Bt sub sp kurstaki* (HD1) to ascertain their insecticidal activity. Individual isolates were streaked on plain luria agar plates and incubated overnight at 37°C. One loop of overnight cultures was inoculated in Luria broth and kept for sporulation under shaking condition at 28°C for 24h.

Leaf dip bioassay method developed by Shelton *et al.* (1993) was adopted for bioassay. Groundnut leaf containing four leaflets was dipped into *Bt* culture broth ( $3.2 \times 10^5$  CFU/1ml) containing 0.2% Triton X-100 for 10 minutes. The dipped leaf was kept for drying. After drying, the petiole of leaf was swabbed with wet cotton to maintain leaf succulence and turgidity. One groundnut leaf was used as one replication, which was placed in a Petri plate. Ten larvae were released per one replication. Likewise three replications were maintained. HD-1 served as a reference strain. The leaf dipped in distilled water served as control. All the *Bt* strains were tested at  $3.2 \times 10^5$  CFU/1ml. The mean per cent larval mortality up to 6 days after treatment was recorded.

## RESULTS AND DISCUSSION

Leaf dip bioassays tend to be more viable than diet incorporation methods that they have the advantage of mimicking natural conditions, avoiding problems with the sporulation of *Bt* spores in artificial diets, and permits a natural feeding behavior of test insects (Navon, 2000).

When the larvae of I and III instar *S. litura* treated with native *Bt* strains, the larvae showed normal activity up to 24 h of inoculation. On the second day the rate of feeding diminished. The larvae became sluggish, turned black with signs of vomiting and diarrhoea. Finally larvae died within 2 to 4 days due to starvation and septicemia. The insecticidal activity of *Bt* is

mainly due to its ability to synthesize parasporal crystal. When susceptible insect ingests  $\alpha$  endotoxins of 130 kDa, they are solubilized and proteolytically digested to yield the active toxin form of molecular weight 60 to 70 kDa polypeptide. The toxin bind to protein receptors in the epithelial insect midgut and produces pores leading to the loss of normal membrane function. As a result epithelial cells lyse and feeding activity is paralyzed, finally insect die due to starvation and septicemia (Luthy and Wolfersberger, 2000).

### A. *Bt* strains isolated from soil samples:

Among 114 *Bt* strains tested against I instar *S. litura* larvae, mortality ranged from 10 to 93.33%. Highest mortality was recorded in HD1 reference strain (93.33%) which was on par with the native *Bt* isolates, 375 (90%) and 416 (86.67%) isolated from Central Telangana and High altitude and Tribal Zone. No mortality was recorded in untreated control. Next best treatments were found to be isolate No. 4, 15, 21, 111 and 206 which cause 80 per cent mortality. Lowest mortality (6.67 %) was recorded in *Bt* strain 137 isolated from Scarce Rainfall Zone.

When *Bt* strains tested against III instar *S. litura*, highest mortality (76.67%) was recorded in HD1 reference strain which was on par with the *Bt* strain 375 (70%). No Mortality was recorded in *Bt* strains 8, 137, 148, 229, 268, 408, 425 and untreated control.

**I. Southern Zone:** Among 29 *Bt* strains isolated from the soils of Southern Zone were tested against I instar *S. litura*, highest mortality was recorded in *Bt* strains 4, 15, 21 (80%) which were on par with the *Bt* strains 12, 91 (76.67%), 25, 44, 58, 83 (73.33%). Lowest mortality was recorded in *Bt* strain 8 (13.33%). In most of the *Bt* strains more

than 50% mortality was recorded (Table 1). Highest mortality (63.33%) of III instar *S. litura* was recorded in *Bt* strains 4, 15 and 21 which were on par with the *Bt* strains 12, 25, 32, 44, 58, 77 and 91 (56.67%). 48.27% (14/29) of *Bt* strains showed more than 50% mortality. However wide variation (0 to 63%) in mortality was observed (Table 1).

**II. Scarce Rainfall Zone:** Twenty strains of this zone were evaluated against I instar *S. litura* and highest mortality was recorded in *Bt* strain No. 111(80%) which was on par with the *Bt* strain 139 (76.67%). Lowest mortality was recorded in *Bt* strain 137 (6.67%). More than 50% mortality was recorded in *Bt* strain 103, 106, 109, 111, 122, 126, 136, 139, 150 and 153. Highest mortality (60%) was recorded against III instar *S. litura* in *Bt* strains 111 and 139 which were on par with *Bt* strains 136 (56.67%), 153 (53.33%) followed by *Bt* strain 106 (50%). Remaining other *Bt* strains have showed less than 50% mortality of III instar *S. litura* larvae.

**III. Krishna Zone.** Fourteen isolates of *Bt* were evaluated against I instar *S. litura* and highest mortality was recorded in *Bt* strain 179 (70%), while lowest mortality was recorded in *Bt* strain 165 (16.67%). Maximum mortality of III instar *S. litura* (53.33%) was recorded in *Bt* strain 179 and very low mortality (3.33%) was recorded by the *Bt* strain 168.

**IV. Godavari Zone:** Among the nine isolates of *Bt*, highest mortality (80%) was recorded against I instar *S. litura* in *Bt* strain 206. Minimum mortality (10%) was recorded in *Bt* strain 229. More than 50% mortality was recorded in *Bt* strains 206 and 242. Highest mortality of III instar *S. litura*, (63.33%) was observed in the *Bt* strain 206 and the remaining *Bt* strains gave less than 50% mortality of III instar *S. litura*..

**V. North coastal Zone:** Among the eleven *Bt* isolates evaluated against I instar *S. litura*, highest mortality (63.33%) was recorded in *Bt* strain 261 which was on par with the *Bt* strain 258 (56.67%). Next best treatment was *Bt* strain 257 (53.33%). Less than 50% mortality was recorded in other isolates. Among all the 11 *Bt* strains isolated from this zone showed less than 50% mortality of III instar *S. litur*. Highest mortality (46.67%) was recorded in *Bt* strain 261.

**VI. Northern Telangana Zone:** Eight isolates of this zone were evaluated against I instar *S. litura* and highest mortality (66.67%) was recorded in *Bt* strain 317 and was on par with the *Bt* strains 285 and 299 (60%). Among eight isolates 60% mortality of III instar *S. litura*, was recorded by the *Bt* strain 281 followed by *Bt* strain 317 (50%). Other *Bt* strains gave less than 50% mortality.

**VII. Southern Telangana Zone:** Highest mortality of I instar *S. litura* (76.67%) was recorded in *Bt* strain 341. Lowest mortality (23.33%) was recorded in *Bt* strain 323. Among the nine isolates tested against III instar *S. litura*, only one *Bt* strain 341 recorded highest mortality of 60 per cent while remaining isolates recorded less than 50% mortality.

**VIII. Central Telangana Zone:** Highest mortality of I instar *S. litura* (90%) compared to all isolates of other eight zones was recorded in *Bt* strain 375. Among five *Bt* strains isolated from this zone, four *Bt* strains shown more than 50% mortality. Highest mortality (70%) was recorded by the *Bt* strain 375 against III instar *S. litura* compared to *Bt* strains isolated from other zones.

**IX. High Altitude and Tribal Zone:** Among the nine isolates tested, highest mortality (86.67%) of I instar *S. litura* was recorded in *Bt* strain 416 (86.67%) which was on par with

the reference *Bt* strain HD1. More than 50% mortality was recorded in *Bt* strains 107, 416 and 422. More than 50% mortality of III instar *S. litura* was shown by the *Bt* strains 416 (66.67%), 405 (56.67%) and 422 (53.33%).

### **B. *Bt* Strains isolated from bacteria infected silkworms:**

Five *Bt* strains (83.33%) out of six isolated from bacteria infected silkworms have recorded more than 50% mortality against I instar *S. litura*. Maximum mortality of III instar *S. litura* (56.67%) was recorded with *Bt* strain 432 followed by 50% mortality in *Bt* strains 434, 440.

### **Categorization of *Bt* strains according to their toxicity levels against *S. litura***

Native *Bt* strains when treated against I instar *S. litura*, maximum of 37.5% (45/120) of *Bt* strains recorded mortality in the range of 51-75% while 10.83% (13/120) of *Bt* strains showed more than 75% mortality (Table 2). When all 114 *Bt* strains were treated against III instar *S. litura*, maximum of 49.16% (59/120) isolates showed mortality in the range of 0-25% and minimum of 8.33% (1/120) of *Bt* strains recorded mortality in the range of 76-100%.

The results of present investigations are in accordance with the findings of Valicente and Barreto (2003) who isolated 3408 strains of *Bt* from 1448 soil samples in 10 Brazilian states. These strains were evaluated against *S. frugiperda* larvae and only 62 % of *Bt* strains recorded mortality between 81% and 100% and 1758 caused no mortality. Highest proportion of efficient strains (larval mortality above 75%) was found from the total isolated per region in the South region (16.6%) followed by Southeast (11.1%), western central (3.1%) and North east region (0.4%).

Chilcott and Wigley (1993) reported that the per cent of isolates obtained from the soil with toxicity against lepidopteran larvae alone ranged from 37 to 88%. Puntambekar *et al.* (1997) also screened different *Bt* strains against certain lepidopteran pests and reported that use of 1018 spores per ml of *Bt* var. *kurstaki* (NCIM 2514) could cause 85 % mortality in neonate larvae of *S. litura* and *Pthorimae operculella*. In agreement with the results of present study Sondos *et al.* (2000) also reported that the newly hatched larvae of *S. littoralis* were most sensitive to *Bt* toxin.

The results of Chatterjee (2008) are also comparable with present study. He has evaluated the efficacy of *Bt* var. *kurstaki* (*Btk.*) at concentration 5500 and 3200 IU/mg against 2<sup>nd</sup> instar larvae of *S. litura* and *H. armigera* under laboratory conditions. *Btkurstaki* at 5500 IU/mg recorded 86.49 and 68.59% mortality of *S. litura* and *H. armigera*. The level of toxicity of the biopesticides decreased with time and in most cases mortality was reduced to less than 10% within 5 days.

Merdan *et al.* (2010) isolated 45 *Bt* strains in Egyptian soils of Cotton cultivations represented by 11 governorates. Only two isolates (Shar.5 and Dkah.1) showed high level of toxicity against *S. littoralis* (80 and 87% mortality). Leaf dip bioassay for 5S in dilution of 2g/l in a commercial formulation of *Bt* var. *kurstaki* and *aizawai* resulted in 100 and 93.7 per cent larval mortality *S. litura*, respectively (Sharma *et al.*, 2001).

The potential isolates have to be further explored for their field efficacies in management of lepidopteran pests.

Table 1: Laboratory evaluation of native *Bt* isolates against I and III instar larvae of *S. litura*

Treatment ( <i>Bt</i> isolate No.)	I instar	III Instar
<b>A. A. Soil samples:</b>		
<b>I. Southern Zone</b>		
<b>Per cent mortality</b>		
1 (4)	80.00 (63.93)	63.33 (53.07)
2 (8)	13.33 (21.14)	0.00 (0.00)
3 (12)	76.67 (61.22)	56.67 (48.85)
4 (15)	80.00 (63.43)	63.33 (52.78)
5 (21)	80.00 (63.93)	63.33 (53.07)
6 (22)	63.33 (52.78)	43.33 (41.15)
7 (25)	73.33 (59.00)	56.67 (48.85)
8 (29)	30.00 (33.00)	6.67 (12.29)
9 (32)	73.33 (59.00)	56.67 (48.93)
10 (36)	66.67 (54.78)	36.67 (37.22)
11 (44)	73.33 (59.00)	56.67 (48.85)
12 (49)	66.67 (54.78)	50.00 (45.00)
13 (52)	50.00 (45.00)	40.00 (39.15)
14 (53)	60.00 (50.77)	40.00 (39.23)
15 (57)	63.33 (52.78)	36.67 (37.22)
16 (58)	73.33 (59.00)	56.67 (48.85)
17 (61)	66.67 (54.78)	50.00 (45.00)
18 (65)	23.33 (28.78)	6.67 (12.29)
19 (67)	70.00 (57.00)	53.33 (46.92)
20 (68)	20.00 (26.07)	6.67 (12.29)
21 (71)	56.67 (48.85)	36.67 (37.22)
22 (76)	60.00 (50.85)	43.33 (41.15)
23 (77)	70.00 (57.00)	53.33 (46.92)
24 (83)	76.67 (61.22)	56.67 (48.85)
25 (87)	63.33 (52.78)	40.00 (39.15)
26 (91)	73.33 (59.00)	56.67 (48.85)
27 (94)	30.00 (33.00)	13.33 (21.14)
28 (95)	53.33 (46.92)	26.67 (31.00)
29 (99)	43.33 (41.15)	13.33 (21.14)
<b>II. Scarce Rain Fall Zone</b>		
30 (103)	60.00 (50.85)	36.67 (37.22)
31 (106)	66.67 (54.78)	50.00 (45.00)
32 (109)	50.00 (45.00)	20.00 (26.57)
33 (111)	80.00 (63.93)	60.00 (50.85)
34 (113)	40.00 (39.15)	13.33 (21.14)
35 (118)	16.67 (23.86)	3.33 (6.14)
36 (121)	40.00 (39.23)	23.33 (28.78)
37 (122)	60.00 (50.85)	40.00 (39.15)
38 (123)	40.00 (39.15)	13.33 (21.14)
39 (126)	53.33 (46.92)	30.00 (33.00)
40 (128)	46.67 (43.08)	13.33 (21.14)
41 (132)	20.00 (26.07)	6.67 (12.29)
42 (134)	23.33 (28.08)	10.00 (18.43)
43 (136)	70.00 (57.00)	56.67 (48.85)
44 (137)	6.67 (12.29)	0.00 (0.00)
45 (139)	76.67 (61.22)	60.00 (50.85)
46 (140)	30.00 (33.21)	6.67 (12.29)

Treatment (Bt isolate No.)	I instar	III Instar
47 (148)	13.33 (21.14)	0.00 (0.00)
48 (150)	56.67 (48.85)	33.33 (35.22)
49 (153)	70.00 (57.00)	53.33 (46.92)
<b>III. Krishna Zone</b>		
50 (165)	16.67 (23.86)	3.33 (6.14)
51 (168)	33.33 (35.22)	16.67 (23.86)
52 (169)	26.67 (31.00)	6.67 (12.29)
53 (171)	50.00 (45.00)	16.67 (23.86)
54 (175)	30.00 (33.00)	6.67 (12.29)
55 (179)	70.00 (57.00)	53.33 (46.92)
56 (182)	56.67 (48.85)	43.33 (41.15)
57 (185)	40.00 (39.15)	20.00 (26.07)
58 (188)	60.00 (50.85)	46.67 (43.08)
59 (190)	20.00 (26.57)	6.67 (12.29)
60 (192)	40.00 (39.15)	16.67 (23.86)
61 (193)	50.00 (45.00)	20.00 (26.07)
62 (195)	60.00 (50.85)	36.67 (37.22)
63 (197)	33.33 (35.22)	10.0 (18.43)
<b>IV. Godavari Zone</b>		
64 (203)	23.33 (28.78)	6.67 (12.29)
65 (206)	80.00 (63.93)	63.33 (52.78)
66 (211)	40.00 (39.15)	26.67 (30.79)
67 (217)	30.00 (33.00)	16.67 (23.36)
68 (224)	40.00 (39.15)	10.00 (18.43)
69 (229)	10.00 (18.43)	0.00 (0.00)
70 (232)	40.00 (39.15)	16.67 (23.36)
71 (233)	26.67 (31.00)	6.67 (12.29)
72 (242)	56.67 (48.85)	36.67 (37.22)
<b>V. North Coastal Zone</b>		
73 (247)	40.00 (39.23)	16.67 (23.86)
74 (252)	43.33 (41.15)	20.00 (26.57)
75 (254)	20.00 (26.07)	3.33 (6.14)
76 (257)	53.33 (46.92)	30.00 (33.00)
77 (258)	56.67 (48.85)	36.67 (37.22)
78 (261)	63.33 (53.07)	46.67 (43.08)
79 (264)	43.33 (41.15)	20.00 (26.57)
80 (265)	40.00 (39.15)	16.67 (23.86)
81 (267)	43.33 (41.15)	23.33 (28.78)
82 (268)	16.67 (23.86)	0.00 (0.00)
83 (270)	43.33 (41.15)	16.67 (23.86)
<b>VI. Northern Telangana Zone</b>		
84 (281)	76.67 (61.22)	60.00 (50.85)
85 (285)	60.00 (50.77)	33.33 (35.22)
86 (289)	46.67 (43.08)	20.00 (26.07)
87 (291)	20.00 (26.07)	3.33 (6.14)
88 (299)	60.00 (50.85)	40.00 (39.15)
89 (307)	53.33 (46.92)	36.67 (37.22)
90 (311)	26.67 (31.00)	10.00 (18.43)
91 (317)	66.67 (54.78)	50.00 (45.00)
<b>VII. Southern Telangana Zone</b>		
92 (323)	23.33 (28.78)	6.67 (12.29)
93 (326)	46.67 (43.08)	26.67 (30.79)

Treatment ( <i>Bt</i> isolate No.)	I instar	III Instar
94 (327)	30.00 (33.00)	13.33 (21.14)
95 (333)	30.00 (33.21)	10.00 (15.00)
96 (336)	46.67 (43.08)	16.67 (23.86)
97 (341)	76.67 (61.22)	60.00 (50.85)
98 (347)	46.67 (43.08)	20.00 (26.57)
99 (349)	53.33 (46.92)	30.00 (33.21)
100 (351)	43.33 (41.15)	23.33 (28.78)
<b>VIII. Central Telangana Zone</b>		
101 (364)	26.67 (31.00)	6.67 (12.29)
102 (371)	70.00 (57.00)	46.67 (43.08)
103 (372)	50.00 (45.00)	40.00 (39.15)
104 (375)	90.00 (75.00)	70.00 (57.00)
105 (376)	60.00 (50.85)	36.67 (37.22)
<b>IX. High Altitude and Tribal Zone</b>		
106 (403)	10.00 (18.43)	0.00 (0.00)
107 (405)	73.33 (59.00)	56.67 (48.85)
108 (408)	10.00 (18.43)	0.00 (0.00)
109 (411)	23.33 (28.78)	6.67 (12.29)
110 (416)	86.67 (68.86)	66.67 (54.99)
111 (422)	70.00 (57.00)	53.33 (46.92)
112 (424)	33.33 (35.22)	20.00 (26.57)
113 (425)	13.33 (21.14)	0.00 (0.00)
114 (426)	36.67 (37.22)	16.67 (2.86)
<b>B. Bacteria infected silkworms:</b>		
115 (431)	60.00 (50.85)	46.67 (43.08)
116 (432)	60.00 (50.85)	56.67 (48.85)
117 (434)	60.00 (50.85)	50.00 (45.00)
118 (440)	56.67 (48.93)	50.00 (45.00)
119 (441)	30.00 (33.00)	16.67 (23.86)
120 (447)	53.33 (46.92)	46.67 (43.08)
121 (HD1)	93.33 (77.71)	76.67 (61.22)
122 (control)	0.00 (0.00)	0.00 (0.00)
S.Em±	2.6	3.08
CD (P=0.05)	7.25	8.59

Values in paranthesis are angular transformed values

**Table 2. Categorization of native *Bt* strains according to their toxicity levels against I and III instar *S. litura***

Per cent of mortality	I Instar		III instar	
	No. of <i>Bt</i> isolates	% of <i>Bt</i> isolates	No. of <i>Bt</i> isolates	% of <i>Bt</i> isolates
<i>Bt</i> Isolates causing mortality between 0-25%	19	15.96	59	49.57
<i>Bt</i> Isolates causing mortality between 26-50%	42	35.29	35	29.41
<i>Bt</i> Isolates causing mortality between 51-75%	45	37.81	24	20.17
<i>Bt</i> Isolates causing mortality between 76-100%	13	10.92	1	0.84

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