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Research Article

Isolation, identification, bioassay and field evaluation of native *Bacillus thuringiensis* strains against *Spodoptera litura* (Fabricius) in groundnut (*Arachis hypogaea*)

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ABSTRACT: A field experiment in a randomized block design was conducted to evaluate 28 *Bacillus thuringiensis* isolates along with a reference strain HD1 and untreated control against *Spodoptera litura* in groundnut. Larval population of *S. litura* per meter row at 3 days after spray (DAS) was lowest (9.0) in plot treated with *Bt* strain 341. Mean per cent reduction of larval population over pretreatment was maximum (56.83%) in HD1 reference strain and it was followed by the *Bt* strain 375 (51.45%). Minimum larval population of *S. litura* (7.0) was observed at 5DAS in HD1 and 375 *Bt* strains. Mean per cent reduction of larvae over pretreatment was maximum (68.32%) in HD1 reference strain followed by *Bt* strain 21 (57.27%). Minimum larval population of *S. litura* (5.0) was recorded at 7DAS in plot treated with *Bt* strains HD1, 375 and 416. Mean per cent reduction of larvae over pretreatment was highest (77.02%) in HD1 reference strain followed by *Bt* strain 375 (74.47%). Per cent leaf damage due to *S. litura* was minimum (12.83%) in plots treated with HD1 reference strain followed by strain 375 (14.06%). Maximum pod yield (3900 kg/ha) was recorded in the plots treated with HD1 reference strain followed by *Bt* strain 375 (3870.0kg/ha).

KEY WORDS: Native *Bacillus thuringiensis* strains spray*, Spodoptera litura,* groundnut

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INTRODUCTION

Tobacco caterpillar *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a polyphagous pest on many agriculturally important crops. Indiscriminate use of chemical insecticides to control this pest leads to resistance to chemical insecticides and cause harmful effects on non target organisms (Rao *et al*., 1999). In recent years, microbial insecticides have become a viable alternative to control lepidopteran pests particularly *S. litura* and *Helicoverpa armigera* Hubner. 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). The *Bt* strains were isolated from nine agroclimatic zones of Andhra Pradesh from soil and bacteria infected silkworms and their toxicity was studied to *S. litura* along with the identification of crystal shape and cry gene profile and reported in this paper.

MATERIALS AND METHODS

Isolation

The sodium acetate selection method (Travers *et al*., 1987) was followed to isolate *Bt* from soil samples. Half a gram of soil sample was added to 10 ml of Luria broth in a 100ml of conical flask. The mixture was kept on a shaker for 4h at 250 rpm at 28°C. The sample was taken and subjected to heat shock at 80°C for 3min. Dilutions were prepared at 10^{-1} to 10^{-5} and 100 µl of each dilution was spread on Luria Bertani agar petriplates. The plates were incubated at 37°C for overnight. Colonies were picked up after comparing with morphological characters (cream colored and have appearance of fried egg like colonies on plate) of reference strains and were purified by repeated four way streaking.

Isolation of *Bt* from bacteria infected silkworms

Bacteria infected silkworm, *Bombyx mori* L. larvae were collected from the sericulture cultural areas of Palamaner division in Chittoor district of Andhra Pradesh.

Dead larvae collected were surface sterilized by dipping in 0.25% sodium hypochlorite for 3 seconds, rinsed in sterile distilled water and crushed in a mortar, diluted 3 times with sterile distilled water and the 100µl suspension was spread with 'L' rod on Luria agar plates, incubated overnight at 37°C in incubator. After 24h, colonies were picked up and gram staining was done. The positive isolates obtained in crystal staining were streaked on to the nutrient agar plates which were incubated at 37°C for 24h and stored at 4°C in refrigerator.

Identification of *Bt*

The isolates obtained from different samples were observed for Gram reaction and presence of crystals was observed with the help of phase contrast microscope.

Gram staining

Gram staining of bacteria was done by following Hucker's method as described by Cappuccino and Sherman (1992). A loop of overnight culture grown on Luria Bertani agar was smeared on a clean glass slide. It was allowed to air dry, heat fixed and then stained with crystalline violet solution for 1 min. The slides were washed with tap water and stained with iodine solution for one minute to fix the dye. The slides were destained with 95% ethanol. The slides were then washed with tap water and counter stained with safranin for two min, again washed with tap water, air dried and observed under microscope. Gram positive cells took violet strain. Gram positive isolates were streaked on T_3 medium for sporulation. After 24h incubation in an incubator crystal protein staining was done as follows.

Crystal protein staining

Crystal protein staining was done according to the protocol described by Sharif and Alaeddinoglu (1988). Smears of cells from *Bt* cultures grown in T_3 medium were heat fixed and dipped for 3 min in a small container with 0.25% Comassie brilliant blue solution. The slide was then washed under tap water, blot dried and observed for dark blue coloured crystals under microscope. The positive isolates in crystal protein staining were streaked on nutrient agar and incubated for 24h at 37°C in incubator. After 24h these cultures were stored at 4°C in a refrigerator for preservation.

Bioassay of native *Bt* isolates against *Spodoptera litura*

One loop of overnight cultures grown on L. B. Agar 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 ($3.2x10^5$ C.F.U/1ml) containing 0.2% Triton X-100 for 10 minutes and allowed to dry. After drying, the petiole of leaf was swabbed with wet cotton to maintain leaf succulence and turgidity. One groundnut leaf was used for one replication, which was placed in a Petri plate. Ten larvae were released per one replication. HD-1 served as a reference strain. The leaf dipped in distilled water served as control. The larval mortality was assessed after 48h at regular intervals.

Bioassay with different concentrations of *Bt* isolates against *S. litura* to determine LC_{50} values

Forty ml Luria broth was taken in conical flask. The isolates with more than 50% mortality (Isolates: 4, 12, 15, 21, 25, 32, 44, 83, 111, 139, 206, 281, 341, 375, 405, 416) in bioassay were taken along with reference strain (HD1) for bioassay to determine LC_{50} values. After cooling, inoculated with one loop of each *Bt* isolate into conical flask containing L.B. broth. Then Luria broth was kept in shaker at 300rpm for 3 days. Serial dilutions were prepared at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. 100µl of each dilution was taken and spread on Luria agar plate with 'L' rod. The plates were kept in an incubator for overnight at 37°C. After 24h colony count was taken. Then x µl of inoculum added for 30ml of water was calculated with the following formula.

$$\frac{1200}{\text{No. of colony count at } 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} \text{ and } 10^{-5} \text{ dilutions}} \times 30$$

x µl of inoculum added to 30 ml of H_2O

To x µl water was added to make up 30ml. Groundnut leaf containing four leaflets were dipped into a culture broth containing 0.2% Triton X-100 for 10 minutes. Then leaf was kept for drying and dipped the leaf in *Bt* strains with different dilutions. One groundnut leaf for one replication was placed in a petriplate. Ten second instar larvae were released. HD-1 served as a reference strain. The leaf dipped in distilled water served as control. The larval mortality was assessed after 48h and LC_{50} values were determined using probit analysis.

Primers (*Cry* 1F, *Cry* 2, *Cry* 8, *Cry* 9, *Cry* 20, *Lep*1, *Lep*2 were used for amplification of *Bt* strains (Table 5). Field experiments were laid out at in randomized block design with 30 treatments and two replications at research farm of Regional Agricultural Station (RARS), Tirupati to evaluate 28 *Bt* isolates which were found to be effective in lab bioassay against *S. litura* on groundnut along with a reference strain HD1 and an untreated control. The soil type was red sandy loam. Plot size of 2 x $2.5m^2$ was employed for each treatment in a replication. All agronomic practices were followed as per the recommendations.

Preparation of *Bt* formulations

Barley based media was used for growth and multiplication of 28 native *Bt* isolates (Vimaladevi *et al*., 2005) along with reference strain HD1. Five grams of powdered barley was taken in a 250ml conical flask. The

remaining ingredients (Yeast extract 63mg, $CaCl_2$ 24mg, $MgSO_4$ 60mg, K_2HPO_4 and KH_2PO_4 50mg were dissolved separately in 50ml distilled water and was added to already prepared barley. The medium was adjusted to pH 7.2. Flasks containing media were sterilized at 15 psi for 20 minutes, cooled and inoculated with 2% (v/v) of *Bt* spore suspension with 3.4 $x10^5$ C.F.U/1ml multiplied on Luria broth and incubated for 48h at 30^0C on a shaker at 200rpm. The medium from flasks was centrifuged, the pellet was dried in a laminar air flow and used for field application.

Suspension containing *Bt* was mixed with a whitener (Ujala) @ 1ml/l as UV protectant, jaggery @ 2 g/l as feeding additive and triton-X @ 2ml/l as emulsifying agent. *Bt* @1g/l was sprayed when Ist instar *S. litura* larva appeared.

The pretreatment data of *S. litura* larva in a meter row of each plot and total number of leaves and damaged leaves from five plants selected at random were recorded. Each treatment was imposed with *Bt* formulation @ 1gm/l. Post treatment counts of larval population per meter row at 3,5 and 7 days after spraying (DAS) were recorded. Mean per cent reduction of larvae over pre-treatment was determined with the following formula.

Mean per cent reduction = (Pretreatment – Post treatment)/(Pre treatment)x100

Pod yield was also recorded after harvest. The data were subjected to statistical analysis (ANOVA).

RESULTS AND DISCUSSION

Of the 410 isolates, 210 were gram positive (Table 1). From these 120 crystal staining positive *Bt* strains identified (Table 1), different shapes of crystals namely spherical, irregular, bipyramidal, cuboidal and rhomboidal were observed (Table 2). Spherical crystals were found to be more compared to others. Thirty one *Bt* isolates (26.66%) out of 120 have recorded more than 50% mortality against all the three instars (I, II and III) of *S. litura* (Table 2).

Probit analysis was done on sixteen native *Bt* strains (4, 12, 15, 21, 25, 32, 44, 83, 111, 139, 206, 281, 341, 375, 405 and 416) which were found effective in preliminary bioassays along with reference strain HD1 that conferred more than 50% of mortality to determine LC_{50}. The larval mortality was found to increase significantly with increase in concentration among all the *Bt* strains tested (Table 2). The reference strain HD1 showed the least LC_{50} value of

Table 1. Number of samples collected and identification of *Bacillus thuringiensis* isolates from different zones of A.P.

S. No	Zone	Isolate number	Total samples	Gram staining positive isolates	Crystal staining positive	
					isolates	Isolate showing more than 50% mortality
I. Soil samples						
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.	4,12,15,21,25,32,44,49,58,61,67,77,83,91
2	Scarce Rainfall Zone	101-164	63	34	103, 106, 109, 111, 113, 118, 121, 122, 123, 126, 128, 132, 134, 136, 137, 139, 140. 148, 150, 153.	106,111,136,139,153
3	Krishna Zone	165-200	34	22	165, 168, 169, 171, 175, 179, 182, 185, 188, 190, 192, 193, 195, 197.	179,
4	Godavari Zone	201-245	44	20	203, 206, 211, 217, 224, 229, 232, 233, 242.	206
5	North Coastal Zone	246-280	34	17	247, 252, 254, 257, 258, 261, 264, 265, 267, 268, 270.	
6	Northern Telangana Zone	281-320	29	15	281, 285, 289, 291, 299, 307, 311, 317.	281,317
7	Southern Telangana Zone	321-355	34	19	323, 326, 327, 333, 336, 341, 347, 349, 351.	341
8	Central Telangana Zone	356-396	40	16	364, 371, 372, 375, 376.	375
9	High Altitude and Tribal Zone	397-429	32	18	403, 405, 408, 411, 416, 422, 424, 425, 426	
	Zone				422, 424, 425, 426	
II. Bacteria infected silkworms from Palamaner division		430-685	255 larvae	21	431, 432, 434, 440, 441, 447	6

Table 2. Bt isolates with more than 50% mean larval mortality against *Spodoptera litura* containing different crystal morphology and *Cry* genes

***Bt* isolate No.**	Isolation source	Crystal shape	Gene composition	Mean mortality percent mortality (%)
I. Southern Zone				
4	Janupuvaripalle,Berceem	Cuboidal	*Cry 2, Cry 20, Lep2*	72.22
12	Gayanavaripalli, Tomato	Cuboidal	*Cry 2, Lep2*	67.78
15	Thummaguntapalle , Ragi	Spherical	*Cry 8, Lep2*	72.22
21	Chinthavaripalle-Sugarcane	Spherical and irregular	*Cry 8, Lep2*	73.33
25	Jammalapalle -Teak	Bipyramidal	*Cry 9, Lep2*	65.56
32	Nallavaripalle- Fallowland	Rhomboidal	*Cry 2, Cry 20, Lep2*	65.56
44	Thimmapuram-Mango	Spherical and Cuboidal	*Cry 9, Lep1, Lep2*	65.56
49	Vanipenta-forest soil	Spherical	*Cry 20*	57.78
58	Kottapalle-cotton crop	Spherical and Cuboidal	*Cry 2, Cry 20, Lep1*	65.56
61	Vanipenta- Turmeric	Spherical	*Lep1*	57.78
67	Bayanapalle- Sunflower	Irregular	*Cry 20, Lep1*	61.11
77	Bheemulapadu - Banana	Irregular	*Lep1, Lep2*	61.11
83	Madharajuguduru- Citrus	Cuboidal	*Cry 2, Cry 9, Lep1*	65.56
91	Tirumala hills	Bipyramidal	*Cry1F,Cry 2, Lep1*	66.67
II. Scarce Rainfall Zone				
106	Mahanandi-Forest soil	Cuboidal	*Cry1F, Cry 20, Lep2*	57.78
111	Mahanandi-Pomogranate	Spherical and irregular	*Cry 2, Cry 9, Lep2*	70.00
136	Panyam- Sapota crop	Spherical and irregular	*Cry 20, Lep1*	63.33
139	Atmakur-Fallow land	Irregular	*Cry 20, Lep1*	70.00
153	Seetharamapuram-Cotton	Spherical	*Cry 2, Lep2*	61.11
179	Bapatla- Cotton (Krishna Zone)	Irregular	*Cry 20, Lep1*	62.22
206	Maruteru-Teak tree (Godavari Zone)	Cuboidal	*Cry1F,Cry 2*	72.22
281	Jagityal-Sunflower (Northern Telangana Zone)	Bipyramidal	*Cry1F, Cry 8, Cry 20, Lep1*	68.89
317	Srirampur- Forest	Bipyramidal	*Cry 1F, Lep2*	57.78
341	Palem-Papaya (Southern Telangana Zone)	Cuboidal	*Cry 1F*	70.00
375	Aswaraopeta -Banana (Central Telangana Zone)	Cuboidal	*Cry 2, Cry 8, Cry 9, Lep2*	80.00
405	Chinthapalli-Coffee (High altitude and Tribal Zone)	Spherical	*Cry 8*	66.67
416	Pandirimamidi-Guava	Rhomboidal	*Lep1*	76.67
422	Seetharampeta-Mango	Spherical	*Cry 1F, Lep1*	61.11
432	Silkworm, B.Kota	Spherical and Cuboidal	*Cry 2, Lep1*	57.78
434	Silkworm, K.V. Palli	Bipyramidal	*Cry 9, Lep1*	53.33
440	Silkworm,Jerurupalli	Rhomboidal	*Lep1*	54.44

0.10 x 10^{-1} CFU/1ml with fiducial limits ranging from 0.63 x 10^{-11} to 0.10 x 10^{-4} CFU/1ml. Among all the native *Bt* strains tested strain 375 exhibited least LC_{50} value of 0.10 x 10^{-6} CFU/1ml with fiducial limits ranging from 0.31 x 10^{-9} to 0.10 x $10^{-4.}$ CFU/1ml (Table 4).

A total of seven lepidopteran specific primers *Cry* 1F, *Cry* 2, *Cry* 8, *Cry* 9, *Cry* 20, *Lep*1 and *Lep* 2 were used in PCR amplification to identify the presence of gene profile of native *Bt* strains. *Bt* strain 375 against *S. litura* recorded maximum mortality

Table 3. Mortality of II instar *Spodoptera litura* at different concentrations of native *Bt* strains

Bt isolates numbers	Per cent larval mortality at different concentrations					
	$1x10^{-1}$ C.F.U/1ml	$1x10^{-2}$ C.F.U/1ml	$1x10^{-3}$ C.F.U/1ml	$1x10^{-4}$ C.F.U/1ml	$1x10^{-5}$ C.F.U/1ml	Mean
4	73.3 (74.35)	70.0 (69.49)	66.67 (64.27)	56.7 (58.77)	53.3 (53.09)	64.0 (60.23)
12	66.67 (65.24)	56.7 (58.78)	53.3 (52.06)	43.3 (45.28)	40.0 (38.64)	52.0 (52.65)
15	80.0 (74.38)	63.3 (68.01)	56.7 (61.06)	53.3 (53.73)	50.0 (46.27)	60.7 (56.50)
21	80.0 (81.39)	76.7 (76.59)	73.3 (71.18)	66.67 (65.23)	56.7 (58.89)	70.7 (65.21)
25	60.0 (59.28)	53.3 (54.36)	50.0 (49.32)	43.3 (44.29)	40.0 (39.36)	49.3 (53.03)
32	56.7 (58.02)	53.3 (52.68)	50.0 (47.28)	40.0 (41.93)	36.7 (36.73)	47.3 (45.99)
44	56.7 (56.02)	50.0 (51.67)	50.0 (47.30)	40.0 (42.96)	40.0 (38.71)	47.3 (50.78)
83	66.7 (64.64)	53.3 (57.44)	50.0 (50.0)	46.7 (42.55)	33.33 (35.36)	50.0 (53.57)
111	70.0 (66.58)	60.0 (61.77)	53.3 (56.78)	50.0 (51.68)	50.0 (46.55)	56.7 (43.36)
139	70.0 (69.13)	63.3 (62.52)	53.3 (55.51)	46.7 (48.33)	43.3 (41.20)	55.3 (41.51)
206	80.0 (79.49)	73.3 (74.30)	70.0 (68.49)	60.0 (62.18)	56.7 (55.53)	68.0 (52.10)
281	70.0 (65.92)	56.7 (59.81)	50.0 (53.43)	46.7 (46.98)	43.3 (40.60)	53.3 (40.16)
341	70.0 (69.77)	63.3 (62.22)	50.0 (54.17)	50.0 (45.94)	36.7 (37.89)	54.0 (40.04)
375	90.0 (88.91)	83.3 (83.99)	76.7 (77.81)	70.0 (70.47)	63.3 (62.17)	76.7 (58.89)
405	56.7 (59.30)	56.7 (55.35)	53.3 (51.34)	50.0 (47.32)	40.0 (43.32)	51.3 (39.47)
416	83.3 (83.34)	76.7 (78.65)	76.7 (73.26)	66.7 (67.26)	60.0 (60.78)	72.7 (55.99)
HD1	93.3 (91.63)	86.7 (87.65)	80.0 (82.50)	76.7 (76.17)	70.0 (68.75)	81.3 (63.01)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

	CD at *P*= 0.05	SEm±
Bt Isolates	0.51	0.19
Concentration	0.28	0.10
Bt Isolates × Concentrations	NS	0.41

compared to all other 120 *Bt* strains may be due to presence of more number of *Cry* genes i.e *Cry* 2, *Cry* 8, *Cry* 9 and *Lep* 2 genes and cuboidal crystals (Table 4).

Although sporulated cultures may be used directly in pest control, *Bt* preparations were processed further to make their physical properties suitable for field application. Such formulations are being sold as either wettable powders or granules or suspension of spores (Bernhard and Utz, 1995).

3 Days After Spraying: Larval population of *S. litura* per meter row was lowest (9.0) in plot treated with *Bt* strain 341 which was on par with the plots treated with *Bt* strains HD1, 139, 206, 416, 375 (9.5 larvae per meter row). Maximum larval population (19.50) was

Table 4. LC_{50} and LC_{90} values of native *Bt* strains against II instar *Spodoptera litura.*

Bt isolates	Regression equation	LC_{50} values C.F.U/1 ml	Fiducial limits CFU/1ml		LC_{90} values C.F.U/1 ml	Fiducial limits C.F.U/1ml		Slope (b)
			Lower	Upper		Lower	Upper	
4	Y = 0.79832 + 0.14415X	0.10×10^{-6}	0.13×10^{-9}	0.40×10^{-3}	0.22×10^{5}	0.91×10^{2}	0.38×10^{13}	0.14415
12	Y = 0.56221 + 0.17015X	0.50×10^{-2}	0.70×10^{-3}	0.23×10^{-1}	0.16×10^{6}	0.74×10^{3}	0.38×10^{12}	0.17015
15	Y = 0.84258 + 0.18722X	0.30×10^{-3}	0.10×10^{-4}	0.15×10^{-2}	0.22×10^{4}	0.42×10^{2}	0.35×10^{8}	0.18722
21	Y = 1.05948 + 0.16694X	0.10×10^{-4}	0.19×10^{-10}	0.10×10^{-3}	0.21×10^{3}	0.05×10^{2}	0.81×10^{7}	0.16694
25	Y = 0.36247 + 0.12648X	0.13×10^{-1}	0.12×10^{-2}	0.21 x 1	0.18×10^{9}	0.18×10^{5}	0.12×10^{26}	0.12648
32	Y = 0.33791 + 0.13537X	0.31×10^{-1}	0.46×10^{-2}	0.69 x 1	0.93×10^{8}	0.18×10^{5}	0.66×10^{22}	0.13537
44	Y = 0.26112 + 0.10959X	0.41×10^{-1}	0.36×10^{-2}	0.08 x 1	0.20×10^{11}	0.13×10^{6}	0.14×10^{42}	0.10959
83	Y = 0.56342 + 0.18781X	0.10×10^{-1}	0.22×10^{-2}	0.45×10^{-1}	0.66×10^{5}	0.54×10^{3}	0.78×10^{10}	0.18781
111	Y = 0.55704 + 0.12870X	0.50×10^{-3}	0.01×10^{-4}	0.37×10^{-2}	0.42×10^{7}	0.18×10^{4}	0.30×10^{21}	0.1287
139	Y = 0.68012 + 0.18047X	0.17×10^{-2}	0.20×10^{-3}	0.71×10^{-2}	0.21×10^{5}	0.20×10^{3}	0.26×10^{10}	0.18047
206	Y = 0.99504 + 0.17118X	0.01×10^{-5}	0.30×10^{-9}	0.20×10^{-3}	0.47×10^{3}	0.10×10^{2}	0.17×10^{8}	0.17118
281	Y = 0.57237 + 0.16202X	0.29×10^{-2}	0.30×10^{-3}	0.14×10^{-2}	0.23×10^{6}	0.76×10^{3}	0.32×10^{12}	0.16202
341	Y = 0.72451 + 0.20658X	0.31×10^{-2}	0.60×10^{-3}	0.10×10^{-1}	0.49×10^{4}	0.10×10^{3}	0.24×10^{8}	0.20658
375	Y = 1.45009 + 0.22802X	0.10×10^{-6}	0.31×10^{-9}	0.10×10^{-4}	0.01×10^{2}	0.25×10^{2}	0.11×10^{3}	0.22802
405	Y = 0.33633 + 0.10089X	0.46×10^{-2}	0.02×10^{-4}	0.10 x 1	0.23×10^{11}	0.80×10^{5}	0.10×10^{54}	0.10089
416	Y = 1.14166 + 0.17359X	0.30×10^{-6}	0.10×10^{-9}	0.02×10^{-4}	0.63×10^{2}	0.02×10^{2}	0.53×10^{6}	0.17359
HD1	Y = 1.60360 + 0.22296X	0.10×10^{-7}	0.63×10^{-11}	0.10×10^{-4}	0.35 x 1	0.63×10^{-1}	0.13×10^{2}	0.22296

recorded in untreated control. Larval population was in the range of 15.0 to 22.0 per meter row before imposing the treatments (Table 5).

Mean per cent reduction of larval population over pretreatment was maximum (56.83%) in HD1 reference strain, and it was on par with the *Bt* strains 375 (51.45%), 416 (50.0%), 21 (49.62%), 206 (48.09%), 4 (47.22%), 15 (46.68%) and 111 (46.54%). Mean per cent reduction of larvae was minimum in *Bt* strain 49 (20.54%).

5 DAS: Minimum larval population of *S. litura* (7.0 larvae per meter row) was observed in HD1 and *Bt* strains 375 which were on par with the *Bt* strains 416 (7.50), 206 (8.0), 21, 341 (8.50), 4, 25, 139 (9.0) and 15 (9.50). Maximum Larval population (21.0) per meter row was recorded in control (Table 5).

Table 5. Field evaluation of native *Bacillus thuringiensis* isolates against *Spodoptera litura* larvae in groundnut

Treatments	Isolates	Pre-treatment	Post treatment					
			Larva/m row			Mean % reduction		
			3 DAS	5 DAS	7 DAS	3 DAS	5 DAS	7 DAS
T_1	4	20	10.5	9	6	47.22 (43.41)	55.05 (47.90)	69.70 (56.63)
T_2	12	22	13	12.5	9.5	40.58 (39.51)	42.96 (40.93)	56.83 (48.93)
T_3	15	21	11	9.5	7	46.68 (43.06)	54.58 (47.63)	66.82 (54.83)
T_4	21	20	10	8.5	5.5	49.62 (44.78)	57.27 (49.20)	72.31 (58.30)
T_5	25	15.5	10	9	7	34.24 (35.44)	41.39 (40.01)	53.15 (46.93)
T_6	32	19.5	13	12	9.5	32.54 (34.51)	37.70 (37.72)	50.40 (45.23)
T_7	44	19.5	13	12	9.5	33.33 (35.26)	38.49 (38.35)	51.59 (45.91)
T_8	49	15	12	11.5	10	20.54 (26.51)	24.11 (28.70)	33.48 (35.34)
T_9	58	21.5	14.5	13.5	10.5	31.36 (33.76)	36.07 (36.74)	49.67 (44.80)
T_{10}	61	18.5	14.5	14	12	21.32 (27.42)	24.26 (29.51)	35.15 (36.36)
T_{11}	67	18.5	13.5	12.5	10	26.90 (31.16)	32.16 (34.29)	45.61 (42.40)
T_{12}	77	18.5	13.5	13	10.5	26.76 (31.11)	29.71 (33.03)	43.53 (41.27)
T_{13}	83	18.5	11.5	11	8.5	37.87 (37.98)	40.64 (39.59)	54.09 (47.35)
T_{14}	91	19.5	13	12	9.5	32.54 (34.51)	37.70 (37.72)	50.40 (45.23)
T_{15}	106	17	13	12.5	10.5	23.26 (28.73)	26.04 (30.46)	38.19 (38.17)
T_{16}	111	22.5	12	11	8	46.54 (43.00)	51.19 (45.68)	64.53 (53.47)
T_{17}	136	18	12.5	11.5	9	30.00 (33.14)	35.63 (36.61)	48.75 (44.26)
T_{18}	139	17.5	9.5	9	6.5	45.07 (42.13)	48.19 (43.96)	62.34 (52.20)
T_{19}	153	16	12	11.5	9.5	24.71 (29.70)	28.04 (31.97)	41.37 (39.88)
T_{20}	179	16	11.5	10.5	8.5	28.04 (31.97)	34.31 (35.86)	46.86 (43.20)
T_{21}	206	18.5	9.5	8	5.5	48.09 (43.89)	56.91 (48.98)	70.29 (56.97)
T_{22}	281	17.5	12.5	11.5	7.5	28.29 (32.10)	33.55 (35.23)	57.07 (49.07)
T_{23}	317	18	14	13.5	11.5	21.88 (27.83)	24.38 (29.43)	36.25 (37.02)
T_{24}	341	16	9.5	8.5	6.5	43.53 (41.27)	46.86 (43.20)	59.80 (50.71)
T_{25}	375	19.5	9.5	7	5	51.45 (45.84)	64.08 (53.18)	74.47 (59.74)
T_{26}	405	20	11.5	11	9	42.68 (40.79)	44.95 (42.10)	55.05 (47.90)
T_{27}	416	19	9.5	7.5	5	50.00 (45.00)	60.56 (51.09)	73.33 (59.09)
T_{28}	422	18.5	13.5	13	10.5	26.76 (31.11)	29.71 (33.03)	43.53 (41.27)
T_{29}	HD1	22	9	7	5	56.83 (48.93)	68.32 (55.77)	77.02 (61.52)
T_{30}	Control	17.5	19.5	21	22.5	-	-	-
	S.Em±	1.44	0.85	0.9	1.22	3.83	3.4	4.2
	CD (P=0.05%)	4.17	2.47	2.6	3.54	11.1	9.86	12.17

Figures in parenthesis are angular transformed values

7 DAS: Minimum larval population of *S. litura* per meter row (5.0) was recorded in plot treated with *Bt* strains HD1, 375 and 416 which were on par with the *Bt* strains 21, 206 (5.50), 4 (6.0), 139, 341 (6.50), 15, 25 (7.0), 281 (7.50), 111 (8.0) and 179, 183 (8.50). Maximum larval population per meter row (22.50) was recorded in untreated control (Table 5).

The results of the present study are in accordance with the Behle *et al*., (1996) who have reported the extension of residual insecticidal activity of *Bt* with casein against *Spodoptera exigua.* Leaves treated with the casein formulation (0.5% w/v) of *Bt* resisted wash off often retaining > 60% original insecticidal activity of unexposed treatments compared with <20% of the original activity for unformulated *Bt* preparations. The casein formulation also provided some protection from light induced degradation.

Jaggery (Jacobs and Sundin, 2001), surfactant Tween 80 (Srivatsava *et al*., 2009) have been used in tank mix to enhance bioefficacy of *Bt* against lepidopterans.

Table 6. Effect of native *Bacillus thuringiensis* isolates on cumulative leaf damage (7 and 15 DAS) caused by *Spodoptera litura* in groundnut

Treatments	Isolate	% leaf Damage		Pod yield (Kg/ha)
		(Pretreatment)	(Post treatment)	
T_1	4	42.88 (40.90)	15.45 (23.15)	3680.0
T_2	12	45.63 (42.49)	21.07 (27.32)	3400.0
T_3	15	40.48 (39.51)	15.57 (23.23)	3640.0
T_4	21	47.35 (43.48)	15.26 (22.99)	3780.0
T_5	25	49.27 (44.58)	24.22 (29.48)	3260.0
T_6	32	38.32 (38.24)	19.98 (26.54)	3140.0
T_7	44	37.87 (37.98)	19.21 (25.95)	3200.0
T_8	49	38.55 (38.34)	25.76 (30.40)	2600.0
T_9	58	39.22 (38.77)	21.30 (27.48)	3020.0
T_{10}	61	40.61 (39.59)	26.92 (31.25)	2660.0
T_{11}	67	36.81 (37.35)	21.99 (27.94)	2940.0
T_{12}	77	36.24 (37.01)	22.54 (28.34)	2840.0
T_{13}	83	39.26 (38.80)	19.19 (25.95)	3300.0
T_{14}	91	42.37 (40.59)	22.64 (28.41)	3080.0
T_{15}	106	41.85 (40.31)	26.67 (31.10)	2800.0
T_{16}	111	48.92 (44.38)	19.54 (26.22)	3600.0
T_{17}	136	57.31 (49.20)	32.57 (34.80)	2980.0
T_{18}	139	45.22 (42.23)	18.77 (25.67)	3500.0
T_{19}	153	36.51 (37.16)	23.15 (28.76)	2820.0
T_{20}	179	41.88 (40.33)	25.60 (30.38)	2960.0
T_{21}	206	49.15 (44.51)	16.95 (24.31)	3740.0
T_{22}	281	39.81 (39.12)	18.07 (25.14)	3440.0
T_{23}	317	40.01 (39.23)	26.10 (30.69)	2740.0
T_{24}	341	41.65 (40.14)	18.63 (25.57)	3440.0
T_{25}	375	49.81 (44.89)	14.06 (22.01)	3870.0
T_{26}	405	49.32 (44.61)	23.59 (29.06)	3320.0
T_{27}	416	49.15 (44.51)	15.02 (22.80)	3820.0
T_{28}	422	36.27 (37.03)	22.42 (28.26)	2880.0
T_{29}	HD1	55.22 (47.99)	12.83 (20.99)	3900.0
T_{30}	Control	35.62 (36.55)	66.38 (54.58)	2480.0
	S. Em±	1.89	1.04	258.9
	CD (*P*=0.05%)	5.48	3.02	794.6
	CV			8.51

According to Vimala Devi *et al*., (2005) yield of castor was higher (1539 g) when *Bt* multiplied on barley medium was sprayed against castor semi looper *Achoea janata* compared to nutrient broth medium (89.10 g) and molasses medium (216.68 g).

Cumulative per cent leaf damage due to *S. litura* at 3 and 7 DAS was minimum (12.83%) in plots treated with HD1 reference strain which was on par with the *Bt* strains 375 (14.06%), 416 (15.02%) , 21 (15.26%), 4 (15.45%) and 15 (15.57%). Per cent leaf damage was maximum (66.38%) in untreated control (Table 6). Per cent leaf damage in pre treatment was in the range of 36.24% to 55.22%, whereas as in post treatment the leaf damage was 12.83% to 66.38%.

Bt was highly effective against lepidopteran larvae of groundnut tested (Jayanthi *et al.,* 1996). Dipel (0.05%) + Chlorpyriphos (0.025%) and Dimilin (0.025%) + Chlorpyriphos (0.025%) were superior and significantly reduced the larval population of *S. litura* by 71.86% and 69.25% respectively on groundnut (Obulpathi *et al*., 2000). According to Loganathan *et al*., (2002) the *Bt* based Spicturin @ 2.0, 1.5, and 1.0 l/ha effectively decreased the *S. litura* larvae on groundnut.

Effectiveness of native *Bt* isolates on the pod yield of groundnut

Maximum pod yield (3900kg/ha) was recorded in the plots treated with HD1 reference strain. It was on par with the *Bt* strains 4, 12, 15, 21, 25, 32, 44, 83, 111, 139, 206, 281, 341, 375, 405 and 416. Minimum yield (2480 kg/ha) was recorded in control (Table 6).

Bt ($1x10^7$/ml) along with fenvalerate (0.005%) resulted in highest larval population reduction of *S. litura*, lowest leaf damage (20.15%) and highest pod yield (15.03g/plant) in groundnut (Jayanthi and Padmavathamma, 2001).

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