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LABORATORY EVALUATION OF *Bacillus thuringiensis* (Berliner) NATIVE TO FOREST ECOSYSTEM AGAINST *Spodoptea litura* (Fabricius)

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ABSTRACT

A total of 61 *Bacillus thuringiensis* strains were collected from 264 soil samples representing different forest ecosystem in Southern zone of Andhra Pradesh in Chittoor, Kadapa and Nellore districts during 2014-2016. These strains were tested for their efficacy against third instar larvae of *Spodoptera litura* in laboratory bioassay studies. A series of 0.00 to 53.33; 0.00 to 20.00; 0.00 to 30.00; 0.00 to 30.00 and 0.00 to 23.33 per cent larval mortality was observed at 72, 96, 120, 144 and 168 hours after treatment, respectively. A cumulative mortality of 0 to 100 per cent was observed among the 61 strains collected from forest ecosystem. The *Bt* strain F493 recorded highest mortality of 100 per cent, followed by F468 (86.67%) which were comparable with standard strain HD-1 for their efficacy. The other strains F287 (76.67%), F504 (76.67%) were proved next best to F493, HD-1 and F468 for their efficacy which were statistically on par with each other.

KEYWORDS: *Bacillus thuringiensis*, bioassay studies, forest soils, *Spodoptera litura*.

INTRODUCTION

Spodoptera litura (Fabricius) is a serious insect pest on groundnut during *rabi* in groundnut growing regions of Andhra Pradesh particularly in Chittoor, Kadapa, Nellore and Anantapur districts. Application of synthetic insecticides is one of the major strategies used by the farmers for managing this pest. The farmers of Chittoor district apply around 4-5 rounds of insecticides including combination insecticides for managing *S. litura*. Brooke and Hines (1999) reported that, 40 per cent of the broad spectrum chemical insecticides have been targeted for the control of lepidopteran pests.

Microbial pesticides are considered as an important component of IPM, among which *Bt* based biopesticides occupies the pivotal position. *Bt* is a Gram-positive bacterium, stands out representing approximately 95 per cent of microorganisms used in biological control of agricultural pests in different countries, which accounts for 1.3 per cent of total pesticides (Ramanujam *et al*., 2014). Though commercial *Bt* strains have been used since ages for managing the lepidopteran pests, there is a need to isolate and test the efficacy of locally available *Bt* strains that can fit into local agro ecosystem.

MATERIAL AND METHODS

The studies were carried out at Regional Agricultural Research Station, Tirupati during 2014-15 and 2015-16. A total of 264 soil samples were collected from forest ecosystem in Southern zone of Andhra Pradesh representing three districts *viz.,* Chittoor, Kadapa and Nellore. Out of 264 soil samples, 61 *B.thuringiensis* strains were isolated and these strains along with standard strain HD-1 were used to determine their activity against *S. litura* in laboratory bioassay. All the 61 strains along with standard strain was inoculated on Luria Bertani agar media and incubated overnight at 37^0C. One loop of overnight cultures was inoculated in Luria broth and kept for sporulation under shaking condition at 28^0 C for 24h. *S. litura* culture was reared till III instar for conducting bioassay studies. The bioassay experiment had 63 treatments with three replications.

Bioassay was followed by leaf dip bioassay method developed by Shelton *et al.* (1993). Groundnut compound leaf containing four leaflets was dipped for 10 minutes into *Bt* culture broth (5×10^8 CFU 1 mL^{-1}) containing 0.2 per cent Triton X-100, then kept leaf for air drying till leaf surface free from moisture. After drying, the petiole of leaf was swabbed with wet cotton to maintain leaf

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succulence and turgidity. Two compound leaves were used for one replication, which was placed in a Petri plate of 9 cm diameter. 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 72h at regular intervals. The data subjected to ANOVA in one way analysis using GENSTAT.

RESULTS AND DISCUSSIONS

A total of 61 strains which were native to forest ecosystem of southern zone of Andhra Pradesh were assessed for their efficacy against third instar larvae of *S. litura*. A series of 0.00 to 53.33; 0.00 to 20.00; 0.00 to 30.00; 0.00 to 30.00 and 0.00 to 23.33 per cent larval mortality was observed at 72, 96, 120, 144 and 168 hours after bioassay. A cumulative mortality of 0.00 to 100 per cent was observed in different strains after 168 hours of bioassay.

Among the 61 strains, F468, with larval mortality of 53.33 per cent was found as effective as standard check HD-1, which was superior over all other strains at 72 hours after bioassay. Next best strains, in the efficacy order were F437 (43.33%), F504 (40.00%), F493 (40.00%), F487 (40.00%) and these strains were statistically on par with each other in their efficacy. The larval mortality after 96 hours after bioassay was 36.67 per cent in F493, followed by F399 (30.00%) which were comparable with standard check HD-1 (36.67%). The other strains, F261, F323, F435 recorded a larval mortality of 20.00 per cent, followed by F468 (16.67%), F500 (13.33%), F462 (13.33%), F493 (13.33%), F399 (13.33%), F347 (13.33%) which were statistically on par with each other at 120h. F262 (30.00%), F268 (30.00%) and F307 (23.33%) recorded higher mortality (on par with each other) at 144h and 168h after bioassay F440 recorded recorded 26.67 per cent larval mortality followed by F254 (23.33%), F258 (20.00%), F281 (23.33%), F287 (20.00%), F307 (20.00%), F486 (20.00%) and F491 (23.33%) which were statistically on par with each other.

A cumulative mortality of 0 to 100 per cent was observed among the 61 strains collected from different forest ecosystem. *Bt* strain F493 recorded a higher mortality of 100 per cent, followed by F468 (86.67%) which were comparable with standard strain HD-1 for their efficacy. The other strains F287 (76.67%), F504 (76.67%) were also next best to F493, HD-1 and F468 for their efficacy which were statistically on par with each other (Table.1).

The present results of 100 per cent larval mortality of *S. litura* with locally available *Bt* strains was in accordance with that of Nariman *et al.* (2009) who reported a higher mortality of 100 per cent and 90 per cent of second instar *Spodoptera littoralis* with two *Bt* strains Ts-5 and As-3 collected from seven governorates of Egypt. Meadows (1993) also reported elevated levels of insecticidal activity against *Galleria mellonella* with *B. thuringiensis* strains isolated from cultivated environments, which are preferred by lepidopteran insects.

Further, Meihiar *et al*. (2015) reported that, forest, beach and cultivated soils had more *B. thuringiensis* strains than uncultivated and interior arid soils. The frequency of *B. thuringiensis* was partially dependant on organic matter and pH content of the soil. A total of 65 per cent of the strains were found to be toxic to *Galleria mellonella.* The most toxic isolate of *B. thuringiensis* was obtained from cultivated area and produced bipyramidal, cuboidal and rectangular inclusions.

In present study, only four strains out of 61 strains recorded mortality in the range of 76-100 per cent against larvae of *S. litura* (Table 2). Similarly, Merdan *et al.,* (2010) reported that, among ten *B. thuringiensis* strains they tested two were potent against *S. littoralis* (Boisduval), another two strains against *Helicoverpa armigera* (Hubner) and six strains were potent against *Pectinophora gossypiella* (Saunders). Contrary to this, Valicente and Barreto (2003) reported a higher frequency of 62 per cent of *Bt* strains recorded mortality between 81 to 100 per cent.

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Table 1. Bioassay of *B. thuringiensis* strains collected from Forest ecosystem against third instar *S. litura* larvae under laboratory conditions

S. No.	Isolate	Per cent mortality					
		72 h	96 h	120 h	144 h	168 h	Cumulative
1	F252	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
2	F254	0.00 (0.00)^{a}	3.33 (6.15)^{abcd}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	23.33 (28.78)^{ef}	26.67 (30.79)$^{d-i}$
3	F256	0.00 (0.00)^{a}	0.00 (0.00)^{a}	3.33 (6.15)^{ab}	10.00 (18.44)$^{b-fg}$	3.33 (6.14)^{ab}	16.67 (23.36)^{def}
4	F258	0.00 (0.00)^{a}	10.00 (18.44)^{efg}	0.00 (0.00)^{a}	3.33 (6.15)^{ab}	20.00 (26.57)^{def}	33.33 (35.22)$^{e-j}$
5	F261	0.00 (0.00)^{a}	0.00 (0.00)^{a}	20.00 (26.07)^{d}	10.00 (15.00)$^{b-f}$	13.33 (17.71)$^{b-f}$	43.33 (41.07)^{ghijk}
6	F262	10.00 (15.00)^{bcd}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	30.00 (33.00)^{h}	6.67 (12.29)^{abcd}	46.67 (43.08)^{hijk}
7	F263	10.00 (18.43)^{cde}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	10.00 (18.43)^{de}
8	F268	0.00 (0.00)^{a}	16.67 (23.86)^{efghi}	0.00 (0.00)^{a}	30.00 (33.00)^{h}	13.33 (21.14)$^{b-f}$	60.00 (50.85)^{jklm}
9	F277	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
10	F281	20.00 (26.07)^{efghi}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	23.33 (28.78)^{ef}	43.33 (41.15)^{ghijk}
11	F284	20.00 (26.07)^{efghi}	6.67 (12.29)^{ade}	0.00 (0.00)^{a}	10.00 (15.00)$^{b-f}$	6.67 (8.86)^{abc}	43.33 (40.78)^{fghijk}
12	F287	20.00 (26.07)^{efghi}	26.67 (30.79)^{hijkl}	0.00 (0.00)^{a}	10.00 (18.44)$^{b-fg}$	20.00 (26.57)^{def}	76.67 (61.22)^{lmn}
13	F297	10.00 (18.43)^{cde}	23.33 (28.78)^{ghijkl}	0.00 (0.00)^{a}	10.00 (15.00)$^{b-f}$	6.67 (12.29)^{abcd}	50.00 (45.00)^{ijkl}
14	F307	0.00 (0.00)^{a}	10.00 (18.44)^{efg}	0.00 (0.00)^{a}	23.33 (28.78)^{gh}	20.00 (26.07)^{def}	53.33 (47.01)^{ijkl}
15	F309	3.33 (6.14)^{ab}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	16.67 (23.86)^{efgh}	6.67 (12.29)^{abcd}	26.67 (31.00)$^{d-i}$
16	F316	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	16.67 (23.86)^{cdef}	16.67 (23.86)^{defg}
17	F321	33.33 (34.93)^{ijklm}	6.67 (12.29)^{abde}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	10.00 (18.43)$^{b-f}$	50.00 (45.08)^{ijkl}
18	F323	13.33 (21.14)^{cdef}	3.33 (6.15)^{abcd}	20.00 (21.15)^{cd}	10.00 (15.00)$^{b-f}$	10.00 (18.43)$^{b-f}$	56.67 (49.22)^{jkl}
19	F328	6.67 (12.29)^{bc}	16.67 (19.22)^{efgh}	6.67 (12.29)^{bc}	10.00 (11.07)$^{a-e}$	10.00 (11.07)^{abcd}	50.00 (45.00)^{ijkl}
20	F339	3.33 (6.14)^{ab}	10.00 (15.00)^{def}	13.33 (17.71)^{cd}	6.67 (8.86)^{abcd}	16.67 (23.86)^{cdef}	50.00 (45.00)^{ijkl}
21	F347	10.00 (18.43)^{cde}	13.33 (21.15)^{efgh}	13.33 (17.71)^{cd}	0.00 (0.00)^{a}	13.33 (17.71)$^{b-f}$	50.00 (44.71)^{ijkl}
22	F361	10.00 (18.43)^{cde}	20.00 (26.07)^{fghij}	10.00 (15.00)^{bcd}	3.33 (6.15)^{ab}	10.00 (15.00)$^{a-e}$	53.33 (47.22)^{ijkl}

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Table 1. Cont...

S. No.	Isolate	Per cent mortality					
		72 h	96 h	120 h	144 h	168 h	Cumulative
23	F396	13.33 (21.14)^{cdef}	10.00 (15.00)^{def}	10.00 (15.00)^{bcd}	10.00 (15.00)$^{b-f}$	6.67 (12.29)^{abcd}	50.00 (45.00)^{ijkl}
24	F399	13.33 (21.14)^{cdef}	30.00 (33.00)^{ijkl}	13.33 (21.15)^{cd}	0.00 (0.00)^{a}	10.00 (15.00)$^{a-e}$	66.67 (54.99)^{klm}
25	F429	0.00 (0.00)^{a}	0.00 (0.00)^{a}	10.00 (18.44)^{cd}	6.67 (12.29)$^{a-e}$	0.00 (0.00)^{a}	16.67 (23.86)^{defg}
26	F430	0.00 (0.00)^{a}	0.00 (0.00)^{a}	6.67 (12.29)^{bc}	0.00 (0.00)^{a}	3.33 (6.14)^{ab}	10.00 (15.00)^{abd}
27	F432	16.67 (23.86)^{defg}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	16.67 (23.86)^{cdef}	33.33 (35.01)$^{e-j}$
28	F433	30.00 (33.00)^{ghijkl}	13.33 (21.15)^{efgh}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	6.67 (12.29) ^{abcd}	50.00 (45.08)^{ijkl}
29	F434	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	10.00 (18.43)$^{b-f}$	10.00 (18.43)^{de}
30	F435	20.00 (26.07)^{efghi}	0.00 (0.00)^{ab}	20.00 (26.07)^{d}	0.00 (0.00)^{a}	10.00 (18.43)$^{b-f}$	50.00 (45.00)^{ijkl}
31	F436	30.00 (33.00)^{ghijkl}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	13.33 (21.14)$^{b-f}$	43.33 (41.15)^{ghijk}
32	F437	43.33 (41.15)^{lmn}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	16.67 (23.86)^{cdef}	60.00 (50.85)^{jklm}
33	F438	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
34	F440	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	26.67 (31.00)^{f}	26.67 (31.00)$^{d-i}$
35	F441	0.00 (0.00)^{a}	10.00 (15.00)^{def}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	10.00 (15.00)^{abcd}
36	F443	26.67 (31.00)^{fghijkl}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	26.67 (31.00)$^{d-i}$
37	F444	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	16.67 (19.93)^{bdefg}	10.00 (18.43)$^{b-f}$	26.67 (30.29)$^{d-i}$
38	F445	20.00 (26.07)^{efghi}	3.33 (6.15)^{abcd}	3.33 (6.15)^{ab}	0.00 (0.00)^{a}	6.67 (8.86)^{abc}	33.33 (35.01)$^{e-j}$
39	F447	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	13.33 (21.15)^{defgh}	0.00 (0.00)^{a}	13.33 (21.14)^{de}
40	F455	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	15.00 (15.00)$^{a-e}$	10.00 (15.00)^{ad}
41	F457	13.33 (21.14)^{cdef}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	3.33 (6.14)^{ab}	16.67 (23.86)^{defg}
42	F459	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
43	F462	33.33 (34.93)$^{h-m}$	16.67 (23.86)^{efghi}	13.33 (21.15)^{cd}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	63.33 (53.07)^{klm}

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Table 1. Cont...

S. No.	Isolate	Per cent mortality					
		72 h	96 h	120 h	144 h	168 h	Cumulative
44	F463	0.00 (0.00)^{a}	3.33 (6.15)^{abcd}	0.00 (0.00)^{a}	10.00 (18.44)$^{b-fg}$	13.33 (21.14)$^{b-f}$	26.67 (30.79)$^{d-i}$
45	F468	53.33 (46.92)^{n}	0.00 (0.00)^{abc}	16.67 (23.86)^{d}	0.00 (0.00)^{a}	16.67 (23.86)^{cdef}	86.67 (72.78)^{no}
46	F482	0.00 (0.00)^{a}	13.33 (21.15)^{efgh}	0.00 (0.00)^{a}	20.00 (26.07)^{fgh}	10.00 (18.43)$^{b-f}$	43.33 (41.07)^{ghijk}
47	F484	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	3.33 (6.15)^{ab}	6.67 (12.29)^{abcd}	10.00 (18.43)^{de}
48	F486	0.00 (0.00)^{a}	13.33 (21.15)^{efgh}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	20.00 (26.57)^{def}	33.33 (35.22)$^{e-j}$
49	F487	40.00 (39.15)^{jlmn}	13.33 (21.15)^{efgh}	0.00 (0.00)^{a}	6.67 (12.29)$^{a-e}$	0.00 (0.00)^{a}	60.00 (50.94)^{jklm}
50	F490	0.00 (0.00)^{a}	26.67 (30.79)^{hijkl}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	6.67 (12.29)^{abcd}	33.33 (34.93)$^{e-j}$
51	F491	13.33 (21.14)^{cdef}	3.33 (6.15)^{abcd}	0.00 (0.00)^{a}	10.00 (18.44)$^{b-fg}$	23.33 (28.78)^{ef}	50.00 (45.00)^{ijkl}
52	F493	40.00 (39.15)^{jklmn}	36.67 (37.23)^{jkl}	13.33 (21.15)^{cd}	10.00 (18.44)$^{b-fg}$	0.00 (0.00)^{a}	100.00 (90.00)^{p}
53	F498	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	13.33 (21.15)^{defgh}	13.33 (21.14)$^{b-f}$	26.67 (30.79)$^{d-i}$
54	F500	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	13.33 (21.15)^{cd}	0.00 (0.00)^{a}	3.33 (6.14)^{ab}	16.67 (23.86)^{defg}
55	F503	0.00 (0.00)^{a}	3.33 (6.15)^{abcd}	0.00 (0.00)^{a}	3.33 (6.15)^{abc}	3.33 (6.14)^{ab}	10.00 (15.00)^{abcd}
56	F504	40.00 (39.15)^{jklmn}	20.00 (26.07)^{fghijk}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	16.67 (23.86)^{cdef}	76.67 (65.85)^{mn}
57	F505	23.33 (28.78)$^{e-j}$	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	10.00 (18.44)$^{b-fg}$	10.00 (18.43)$^{b-f}$	43.33 (41.15)^{ghijk}
58	F506	23.33 (24.15)^{defgh}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	3.33 (6.15)^{abc}	0.00 (0.00)^{a}	26.67 (26.07)^{defgh}
59	F508	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{ab}
60	F510	10.00 (15.00)^{bcd}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	6.67 (12.29)^{abcd}	16.67 (23.86)^{defg}
61	F514	23.33 (28.78)$^{e-jk}$	10.00 (18.44)^{efg}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	33.33 (35.22)$^{e-j}$
62	HD1	50.00 (45.00)^{mn}	36.67 (37.23)^{jl}	3.33 (6.15)^{ab}	6.67 (12.29)$^{a-e}$	0.00 (0.00)^{a}	96.67 (83.86)^{op}
63	Control	0.00 (0.00)^{a}	0.00 (0.00)^{abc}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{abc}
	F.Pr.	<.001	<.001	<.001	<.001	<.001	<.001
	SeD	4.54	4.94	4.78	5.62	6.25	7.10
	LSD	8.98	9.77	9.46	11.13	12.37	14.06

Figures in parenthesis are angular transofrmed values.
Values followed same letter are not significantly different as per DMRT.

Table 2. Cumulative mortality range of *B. thuringiensis* strains native to forest ecosystem based on their efficacy against *S. litura*

S. No	Mortality range	Strains		
		ID	No. of Strains	Frequency
1	Strains with 0-25 % mortality	F252, F263, F277, F316, F429, F430, F434, F438, F441, F447, F455, F457, F459, F484, F500, F503, F508, F510	18	29.51
2	Strains with 26-50 % mortality	F254, F256, F258, F261, F262, F281, F284, F297, F307, F309, F321, F328, F339, F347, F361, F396, F432, F433, F435, F436, F440, F443, F444, F445, F463, F482, F486, F490, F491, F498, F505, F506, F514	33	54.10
3	Strains with 51-75 % mortality	F268, F323, F399, F437, F462, F487	6	9.84
4	Strains with 76-100 % mortality	F287, F468, F493, F504	4	6.56
		Total	61	100.00

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