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Field evaluation of native *Bacillus thuringiensis* strains against *Helicoverpa armigera* (Hubner) in field bean (*Lablab purpureus*)

C. LALITHA, T. MURALIKRISHNA and V. SRINIVASA RAO

Department of Entomology, Regional Agricultural Research Station, Acharya N. G. Ranga Agricultural University, Tirupati-517 502, Andhra Pradesh, India.
E mail: lalithachallakadapa@gmail.com

ABSTRACT : A field experiment was conducted in a Randomized Block Design to evaluate 28 native isolates of *B.t.* along with a reference strain HD1 and untreated control against *Helicoverpa armigera* in Field bean. Solid and liquid formulations of native *B.t* strains were tested against *H. armigera* and it was found that larval population and per cent pod damage were significantly less in solid formulation compared to liquid formulation. Field bean pod yield (kg/ha) was more in solid formulation compared to liquid formulation. *B.t* strain 281 resulted in minimum larval population and pod damage by *H. armigera* and maximum healthy green pod yield.

Keywords : *Bacillus thuringiensis*, Field bean, *Helicoverpa armigera*,.

INTRODUCTION

Field bean or lablab bean (*Lablab purpureus* (L.) Sweet) is an important pulse and vegetable crop cultivated for its tender and mature pods, seeds and also for fodder. The crop is attacked by as many as 55 species of insects. Among them, the pod borers are considered to be the most devastating pests causing pod loss to the tune of nearly 54 per cent. Some of the effective *B.t.* strains based on preliminary bioassay against *H. armigera* were selected for field evaluation to know the effective strains against *H. armigera* in Field bean crop. Isolation of native *B.t.* strains from different zones of A.P. was conducted to develop effective *B.t.* formulations than already available in the market. As *B.t.* is heat sensitive, direct exposure to sun light loses its persistence in short time. So inorder to increase its persistence solid formulations are developed with barley powder. The present study was conducted to test the efficacy of these formulations.

MATERIALS AND METHODS

Field experiments were laid out in a Randomized Block Design with 30 treatments and two replications at Regional Agricultural Research Station (RARS), Tirupati, Andhra Pradesh to evaluate 28 native isolates of *B.t.* which were found to be effective in lab bioassay against *H. armigera* on field bean along with a reference strain HD1 and an untreated control. The soil type was red sandy loam. Plot size of 2 x 1.35m^2 was employed for each treatment in a replication. All agronomic practices were followed as per the recommendations. Solid and liquid formulations were prepared with spore suspension of 3.4 x10^5 cfu/1ml.

Preparation of solid formulations

Barley based media was used for growth and multiplication of 28 native *B.t* isolates (Vimaladevi *et al*., 2005) along with reference strain HD1. Powdered barley (5g) 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 this was added to already prepared barley. pH of the medium was adjusted to 7.2. Flasks containing media were sterilized at 15 psi for 20 minutes, cooled and inoculated with 2% (v/v) of *B.t* spore suspension with 3.4 x10^5 cfu/1ml multiplied on Luria broth and incubated for 48h at 30^0C on a shaker at 200 rpm. The medium from flasks was centrifuged the pellet was dried in a laminar air flow and used for field application.

Preparation of liquid formulations

MGM broth (40ml) was taken in a 250 ml conical flask. Flasks containing media were sterilized at 15psi pressure for 20 minutes, cooled and inoculated with native *B.t.* isolates, along with reference strain (HD1) and incubated for 72h in a shaker at 200 rpm. This medium was taken at the rate of 2ml/l was used for field application. Solid and liquid formulations were prepared with spore suspension of 3.4 x10^5 cfu/ml.

Suspension containing *B.t.* was mixed with ujala (1ml/l) as UV protectant, jaggery (2 g/l) as feeding additive and triton-X (2ml/l) as emulsifying agent. The formulation (1g/l) was sprayed when *H. armigera* larvae appeared and the pod damage exceeded 15 per cent at 60 days after sowing. Treatment details with different *B.t.* strain numbers are given in Table 1.

In each plot, pre treatment data of *H. armigera* larvae, total number of pods and damaged pods were recorded from five plants selected at random. Each treatment was imposed with *B.t.* formulation at the rate of 1gm/lit. Post treatment counts of larval population from five randomly selected plants 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.

$$\text{Mean per cent reduction} = \frac{\text{Pre-treatment} - \text{Post treatment}}{\text{Pre treatment}} \times 100$$

Number of damaged pods and total number of pods for 5 randomly selected plants were recorded at 7 and 15 days after spraying and pod yield was also recorded after harvest in kg per hectare. The data were subjected to statistical analysis (ANOVA). The results of the field experiment conducted with both solid and liquid formulation of native 28 *B.t.* isolates were presented here under

RESULTS AND DISCUSSION

Solid formulation: Larval population per 5 plants at 3 DAS was lowest (2.0) in plot treated with *B.t.* strain 281 and HD1. Mean per cent reduction of *H. armigera* larvae over pre treatment was maximum (64.58%) in HD1 reference strain followed by *B.t.* strain 281 (61.67%). Minimum larval population (1.50) per five plants was observed at 5DAS in HD1 and *B.t.* strain 281. Mean per cent reduction of *H. armigera* larvae over pre treatment was maximum (79.17%) in HD1 reference strain followed by *B.t.* strain 281 (71.67). Minimum larval population per 5 plants (1.0) at 7DAS was recorded in plot treated with HD1 and *B.t.* strain 281. Mean per cent reduction over pre treatment was highest (85.42%) in HD1 reference strain followed by *B.t.* strain 22 (76.19%) (Table1). Pod damage at 7DAS was minimum (6.01%) in plots treated with HD1 reference strain followed by *B.t.* strain 281 (7.93%) (Table 3). Maximum green pod yield (4703.7kg/ha) was also recorded in plots treated with HD1 reference strain followed by *B.t.* strain 281 (4629.6kg/ha) (Table 4).

The results of present investigations are in accordance with the following authors. Although sporulated cultures may be used directly in pest control. *B.t.* preparations are 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). Inorder to increase the persistence of *B.t.*, jaggery, phenols and flavonoids have been used in tank mix (Jacobs and Sundin, 2001). Srivatsava *et al*. (2009) added certain amendments like natural oils, clay, flour, surfactant (Tween 80), dispersants (cellulose), light blockers (lignin), stickers (pregelatinized) added to enhance bioefficacy of *B.t.* against lepidopterans, coleopterans and dipterans. According to Vimala Devi *et al*., (2005) yield of castor was higher (1539 gm) when *B.t.* multiplied on barley medium was sprayed against castor semi looper *Achoea janata* compared to nutrient broth medium (89.10 gm) and molasses medium (216.68 g). The cost of production was also less in barley medium compared to others.

Liquid formulation: Minimum larval population (2.50) was recorded at 3 DAS in plots treated with *B.t.* strains HD1, 95, 122, 281. Mean per cent reduction of larval population over pre treatment was highest (61.90%) in HD1 reference strain followed by *B.t.* strain 281 (55.0%). At 5 DAS minimum larval population (2.0 per 5 plants) was recorded in plots treated with native *B.t.* strain 122, 281 and HD1 followed by *B.t.* strains 22, 95, 139 (2.50). Highest mean per cent reduction of *H. armigera* (69.05%) over pre treatment was recorded in HD1 followed by *B.t.* strain 281 (65.0%). Minimum larval population (1.50 per 5 plants) was recorded at 7 DAS in *B.t.* strains HD1, 122, 281. Highest mean per cent reduction (76.19%) of *H. armigera* larvae over pre treatment was recorded in HD1 reference strain followed by *B.t.* strain 281 (71.67). Lowest mean per cent reduction (10.0) was recorded in *B.t.* strain 341 (Table 2). Pod damage due to *H. armigera* at 7 days after treatment was less in plot treated with *B.t.* strain HD1 (8.08%) followed by *B.t.* strain 281(8.33%) (Table 3). Maximum green pod yield (4592.6 kg/ha) was recorded in HD1 strain followed by *B.t.* strain 122 (4333.3kg/ha) (Table 4). All plots treated with native *B.t.* strains in solid formulation recorded more yield compared to liquid formulation. When the *B.t.* strain 281 was sprayed yield in solid formulation (4629.6 kg/ha) was more than that of liquid formulation (4444.4 kg/ha) (Table 3).

Overall in all the plots treated with *B.t.* strains in solid formulation larval population and per cent pod

Table 1. Field evaluation of native *B.t.* isolates (solid formulation) against *Helicoverpa armigera* larvae on field bean

Treatments	Isolate No.	Pre treatment count larvae/5 plants	Post treatment Mean reduction (%)		
			3 DAS	5 DAS	7 DAS
T_1	12	7.00	27.08 (30.93)	29.17 (32.63)	50.0 (45.00)
T_2	22	6.50	53.57 (47.05)	69.05 (56.21)	76.19 (61.26)
T_3	25	5.00	10.00 (13.28)	10.00 (13.28)	40.00 (38.67)
T_4	44	5.50	53.33 (46.98)	63.33 (52.75)	73.33 (59.09)
T_5	53	6.50	45.24 (42.19)	60.71 (51.34)	69.05 (56.21)
T_6	58	5.50	28.33 (31.66)	28.33 (31.66)	46.67 (43.02)
T_7	65	5.50	46.67 (43.02)	55.00 (47.88)	63.33(52.75)
T_8	71	5.00	12.50 (15.00)	20.83 (27.05)	41.67 (40.13)
T_9	83	5.50	36.67 (37.25)	36.67 (37.25)	55.00 (47.88)
T_{10}	87	6.50	46.43 (42.95)	61.90 (51.92)	69.05 (56.21)
T_{11}	91	5.50	26.67 (30.91)	26.67 (30.91)	45.00 (42.12)
T_{12}	95	5.50	45.00 (42.12)	55.00 (47.88)	63.33 (52.75)
T_{13}	106	7.00	20.83 (27.05)	27.08 (30.93)	43.75 (41.38)
T_{14}	122	7.00	56.25 (48.62)	70.83 (57.37)	79.17 (62.95)
T_{15}	139	7.00	41.67 (40.13)	47.92 (43.75)	62.50 (52.50)
T_{16}	153	6.50	32.14 (33.60)	32.14 (33.60)	53.57 (47.05)
T_{17}	169	6.00	10.00 (13.28)	17.14 (24.39)	41.43 (40.06)
T_{18}	182	5.50	25.00 (22.50)	26.67 (30.91)	43.33 (40.65)
T_{19}	193	4.50	10.00 (13.28)	10.00 (13.28)	30.00 (25.38)
T_{20}	206	6.50	38.10 (38.08)	46.43 (42.95)	61.90 (51.92)
T_{21}	281	5.50	61.67 (52.57)	71.67 (58.34)	81.67 (64.67)
T_{22}	285	4.00	12.50 (15.00)	12.50 (15.00)	37.50 (37.50)
T_{23}	299	4.50	10.00 (13.28)	10.00 (13.28)	32.50 (34.62)
T_{24}	317	5.00	30.00 (32.90)	30.00 (32.90)	60.00 (50.77)
T_{25}	341	7.00	0.00 (0.00)	6.25 (10.35)	27.08 (30.93)
T_{26}	375	7.00	37.50 (37.50)	43.75 (41.38)	56.25 (48.62)
T_{27}	408	7.00	8.33 (12.05)	14.58 (22.40)	35.42 (36.51)
T_{28}	422	4.50	12.50 (15.00)	25.00 (22.50)	42.50 (40.38)
T_{29}	HD1	7.00	64.58 (53.49)	79.17 (62.95)	85.42 (67.60)
T_{30}	Control	6.50	-	-	-
	S.Em±	0.70	10.00	8.20	7.29
	CD(P=0.05)	2.02	28.96	23.76	21.1

Values in parenthesis are angular transformed values.

Table 2. Field evaluation of native *B.t.* isolates (liquid formulation) against *H. armigera* larvae on field bean

Treatments	Isolate No	Pre treatment count larvae/ 5 plants	Post treatment Mean reduction (%)		
			3 DAS	5 DAS	7 DAS
T_1	12	5.50	28.33 (31.66)	28.33 (31.66)	36.67 (37.25)
T_2	22	5.50	46.67 (43.02)	55.00 (47.88)	65.00 (54.22)
T_3	25	6.50	7.14 (11.10)	15.48 (23.15)	22.62 (28.20)
T_4	44	7.00	47.92 (43.75)	56.25 (48.62)	58.33 (49.87)
T_5	53	7.00	43.75 (41.38)	50.00 (45.00)	54.17 (47.63)
T_6	58	5.50	26.67 (30.91)	26.67 (30.91)	36.67 (37.25)
T_7	65	6.50	45.00 (42.12)	45.00 (42.12)	51.25 (45.74)
T_8	71	5.50	10.00 (13.28)	18.33 (25.33)	26.67 (30.91)
T_9	83	5.00	29.17 (32.63)	37.50 (37.50)	41.67 (40.13)
T_{10}	87	6.00	50.00 (45.00)	50.00 (45.00)	58.33 (49.87)
T_{11}	91	7.50	19.64 (26.10)	26.79 (31.16)	33.93 (35.45)
T_{12}	95	4.00	37.50 (37.50)	37.50 (37.50)	50.00 (45.00)
T_{13}	106	4.50	12.50 (15.00)	22.50 (28.28)	32.50 (34.62)
T_{14}	122	5.00	50.00 (45.00)	60.00 (50.77)	70.00 (57.10)
T_{15}	139	6.50	36.90 (36.60)	61.90 (51.92)	45.24 (42.19)
T_{16}	153	7.00	29.17 (32.63)	29.17 (32.63)	39.58 (38.17)
T_{17}	169	6.50	7.14 (11.10)	15.48 (23.15)	22.62 (28.20)
T_{18}	182	4.50	20.00 (19.62)	20.00 (19.62)	32.50 (34.62)
T_{19}	193	7.00	6.25 (10.35)	6.25 (10.35)	14.58 (22.40)
T_{20}	206	4.50	32.50 (34.62)	32.50 (34.62)	45.00 (42.12)
T_{21}	281	5.50	55.00 (47.88)	65.00 (54.22)	71.67 (58.34)
T_{22}	285	5.00	10.00(13.28)	10.00 (13.28)	20.00 (26.57)
T_{23}	299	5.50	8.33 (12.05)	8.33 (12.05)	18.33 25.33)
T_{24}	317	6.50	23.81 (28.74)	32.14 (33.60)	46.43 (42.95)
T_{25}	341	5.50	8.33 (12.05)	8.33 (12.05)	10.0 (13.28)
T_{26}	375	5.50	26.67(30.91)	35.00 (35.78)	43.33 (40.65)
T_{27}	408	5.00	8.33 (12.05)	12.50 (15.00)	20.83 (27.05)
T_{28}	422	5.50	10.00 (13.28)	18.33 (25.33)	28.33 (31.66)
T_{29}	HD1	6.50	61.90 (51.92)	69.05 (56.21)	76.19 (61.26)
T_{30}	Control	5.50	-	-	-
	S.Em±	0.66	9.67	7.72	5.99
	CD(P=0.05)	1.93	28.01	22.36	17.36

Values in parenthesis are angular transformed values.

Table 3. Effect of native *B.t.* isolates on pod damage and green pod yield (kg/ha) of field bean

Treatments	Isolate No.	Solid formulation		Liquid formulation	
		Pod Damage (%)	Yield (kg/ha)	Pod Damage (%)	Yield (kg/ha)
T_1	12	13.50 (21.56)	2814.8	20.26 (26.74)	2629.6
T_2	22	9.81 (18.25)	4444.4	12.58 (20.77)	4259.3
T_3	25	18.29 (25.32)	2037.0	19.41 (26.11)	1814.8
T_4	44	9.00 (17.45)	4370.4	12.13 (20.29)	4222.2
T_5	53	9.45 (17.88)	4148.1	10.63 (19.00)	4074.1
T_6	58	15.54 (23.22)	2777.8	18.26 (25.29)	2592.6
T_7	65	13.49 (21.54)	3925.9	14.48 (22.37)	3888.9
T_8	71	15.72 (23.35)	2185.2	17.47 (24.70)	1963.0
T_9	83	18.37 (25.37)	3037.0	15.20 (22.92)	2925.9
T_{10}	87	11.59 (19.90)	4259.3	10.72 (19.08)	4148.1
T_{11}	91	17.59 (24.74)	2629.6	16.95 (24.30)	2481.5
T_{12}	95	13.28 (21.36)	3777.8	14.00 (21.97)	3666.7
T_{13}	106	19.75 (26.38)	2444.4	20.51 (26.93)	2240.7
T_{14}	122	9.40 (17.86)	4444.4	10.68 (19.06)	4333.3
T_{15}	139	14.36 (22.26)	3518.5	13.76 (21.77)	3444.4
T_{16}	153	16.75 (24.15)	2888.9	19.06 (25.88)	2777.8
T_{17}	169	18.91 (25.77)	2111.1	17.39 (24.64)	1888.9
T_{18}	182	18.33 (25.35)	2407.4	18.07 (25.15)	2111.1
T_{19}	193	20.27 (26.76)	1814.8	18.65 (25.54)	1666.7
T_{20}	206	12.52 (20.71)	3444.4	17.50 (24.72)	3333.3
T_{21}	281	7.93 (16.33)	4629.6	8.33 (16.76)	4444.4
T_{22}	285	19.68 (26.33)	1963.0	23.13 (28.74)	1740.7
T_{23}	299	22.70 (28.42)	1888.9	17.91 (25.04)	1703.7
T_{24}	317	11.63 (19.94)	3370.4	12.97 (21.11)	3222.2
T_{25}	341	20.17 (26.68)	1777.8	17.72 (24.87)	1592.6
T_{26}	375	14.78 (22.60)	3222.2	16.08 (23.64)	3074.1
T_{27}	408	18.24 (25.28)	1963.0	17.90 (25.03)	1740.7
T_{28}	422	15.08 (22.84)	2259.3	17.36 (24.61)	2074.1
T_{29}	HD1	6.01 (14.18)	4703.7	8.08 (16.50)	4592.6
T_{30}	Control	45.36 (42.33)	1704.2	49.55 (44.74)	1555.6
	S.Em±	0.75	276.6	0.91	244.2
	CD (P=0.05)	2.16	799.9	2.62	706.2
	C.V. (%)		9.12		8.5

damage was less compared to liquid formulation. Healthy pod yield (kg/ha) was more in solid formulation compared to liquid formulation.The results of present investigations are in accordance with those of Shilpa (2005) who reported that the native isolate P1 recorded highest yield per plot of 7.30 q/ha which was on par with HD1, followed by the isolates D1(6.82 q/ha) and PP10 (6.30 q/ha). *B.t.*-42 and S 13 recorded lowest yield in Chickpea. However Chickpea grain yield was significantly higher in quinolphos treated plot (11.02q/ha) followed by *B.t.* commercial formulation dipel (9.4q/ha) and HD1 (7.8q/ha). *B.t* sub sp. *kurstaki* ($176x10^2$ spores/ml) @ 750ml/ha effectively reduced larvae of *H. armigera* on Chickpea and increased the yield (Kulat *et al.*,1999). According to Glare and Callaghan (2000), dipel (*B.t kurstaki*) is very efficient in the control of *H. armigera* and other lepidopteran pests, but has low activity against *Spodoptera* sp.

Based on the finding, it can be concluded that solid formulation is more effective than liquid formulation and *B.t.* strain 281 was the most effective native strain.

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