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Entomopathogenic screening of native *Bacillus thuringiensis* (Berliner) isolates against *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) under laboratory condition

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Abstract

Fall Armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae) is native to the tropical and subtropical region of America. Being a polyphagous pest known to cause major damage to economically important cultivated grasses. This insect is one of the most important pests of maize, being firstly registered in the India in 2018 and it causes a 33% yield loss in the plant production. The use of chemical pesticides as a prophylactic method causes some problems such as ecological instability, pollution, high costs and death of natural enemies. In the current study, screening of native *B. thuringiensis* isolates and their insecticidal activities were tested against the 2nd instar larvae of maize fall armyworm, *Spodoptera frugiperda* under laboratory condition. Preliminary bioassay was conducted using diet incorporation method and treated with spore crystal lysates prepared from the native *Bacillus thuringiensis* isolates. Upon screening 50.0 per cent (of the *B. thuringiensis* isolates exhibited mortality in the range of 26-50 per cent while 10.0 per cent of the *B. thuringiensis* isolates showed more than 75 per cent mortality after 7 days of exposure. Among the seventy isolates, Bt-Oa1 and Bt-257 recorded maximum of 86.84% mortality after 7 days after treatment.

Keywords: Maize fall armyworm, *Spodoptera frugiperda*, *Bacillus thuringiensis*, Indigenous strains, Insecticidal activity

Introduction

The fall armyworm, *Spodoptera frugiperda* (Smith) (Noctuidae: Lepidoptera), is a polyphagous pest that feeds on 353 plant species belonging to 76 families and causes significant loss in crop production. The larvae feed on several plant species viz., maize, rice, sorghum, sugarcane, cabbage, beet, peanut, soybean, alfalfa, onion, tomato, potato and cotton. Among these host plants, maize and sorghum are most preferred by *S. frugiperda*. The fall armyworm is native to the Americas. This pest is found in most parts of the Western Hemisphere, from southern Canada to Chile and Argentina (Pogue, 2002; Nagoshi, 2007; Bueno *et al.*, 2010) [18, 15, 2]. The incursion of fall armyworm as an invasive pest into Asia was reported for the first time from India on maize during May 2018 (Sharanabasappa *et al.* 2018a). Since then, it has spread to different states of India on maize (Mahadevaswamy *et al.* 2018; Sharanabasappa *et al.* 2018b) [21-22]. The spread of this pest to other Asian countries, including Thailand, Sri Lanka, Bangladesh, Myanmar, Vietnam, Laos, and China (Guo *et al.*, 2018; Wu *et al.*, 2019; NATESC 2019, b; CABI 2019) [9, 25, 16, 3] has occurred quickly. Maize is a staple crop in India, grown in an area of 9.47 million ha with a production of 28.72 million tons per yr. Among the major maize producing states, Karnataka stands first with an area of 1.22 million ha and a production of 3.31 million tons (Anonymous 2017) [1]. The recent invasion of fall armyworm threatens the food security of India. At present, the Central Insecticide Board and Registration Committee recommends the use of chlorantraniliprole 18.5 SC, thiamethoxam 12.6% + lambda cyhalothrin 9.5% ZC, and spinetoram 11.7 SC (DPPQS 2019) [6] for fall armyworm management. For the management of introduced fall armyworm, farmers have resorted to 2 to 3 sprays of different insecticides without the knowledge of their efficacy, because of multiple sprays of insecticides may lead to the quick development of resistance as has occurred in other areas (Gutierrez-Moreno *et al.*, 2019) [10].

Since, *S. frugiperda* was evolving resistance against synthetic insecticides there is a need for alternative strategies including the use of biopesticides.

Microbial biopesticides play a key role in sustainable agriculture. The two main benefits of microbial biopesticides are target specificity and environmental safety (Perez-Garcia *et al.*, 2011) [17]. Among the microbial pathogens, *Bacillus thuringiensis* (Berliner) is the most widely used biopesticides in the world. Compared to the use of chemical control agents, *B. thuringiensis* has many advantages because the parasporal bodies produced by the bacterium at the time of sporulation are highly specific to the target insect pests.

B. thuringiensis is a ubiquitous, Gram positive, aerobic, spore-forming bacterium that synthesizes many entomocidal toxins, among them the crystal endotoxins (Cry) synthesized during sporulation have practical significance. About 90 per cent of the microbial biopesticides currently available on the market are based on *B. thuringiensis* (Kumar and Singh, 2015) [11]. Although biopesticide use at a global scale is increasing by almost 10 per cent every year, it appears that the global market must increase further in the future if these pesticides are to play a visible role as substitutes for chemical pesticides (Damalas and Koutrobas, 2018) [5].

Under biointensive and organic farming conditions, the scope of using sprayable formulations of *B. thuringiensis* is bright. However, the right selection of indigenously isolated *B. thuringiensis* is essential to manage the invasive pest effectively. Keeping the importance of *Bt* in view, native *B. thuringiensis* isolates were screened their insecticidal activities against the 2nd instar larvae of maize fall armyworm.

Materials and Method

Laboratory studies were carried out during 2018 to 2019 at ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Hebbal, Bengaluru. Indigenous seventy *B. thuringiensis* (*Bt*) isolates such as Bt-15, Bt-21, Bt-23, Bt-26, Bt-27, Bt-28, Bt-30, Bt-31, Bt-34, Bt-35, Bt-37, Bt-38, Bt-39, Bt-40, Bt-41, Bt-43, Bt-44, Bt-47, Bt-54, Bt-72, Bt-83, Bt-88, Bt-90, Bt-92, Bt-103, Bt-104, Bt-105, Bt-106, Bt-107, Bt-108, Bt-109, Bt-110, Bt-111, Bt-113, Bt-115, Bt-117, Bt-118, Bt-122, Bt-126, Bt-129, Bt-132, Bt-138, Bt-139, Bt-142, Bt-146, Bt-147, Bt-151, Bt-190, Bt-201, Bt-202, Bt-212, Bt-216, Bt-237, Bt-238, Bt-240, Bt-246, Bt-247, Bt-248, Bt-251, Bt-256, Bt-257, Bt-258, Bt-259, Bt-265, Bt-267, Bt-268, Bt-272, Bt-275, Bt-278 and Bt-Oa1 were maintained at ICAR-NBAIR were used to screen their entomopathogenic potential against second instar larvae of maize fall armyworm. *B. thuringiensis* subsp. *kurstaki* HD-1, obtained from BGSC, Columbus, USA was used as a standard check.

Preparation of spore-crystal formulations of *B. thuringiensis* isolates.

Crude protein extraction from *B. thuringiensis* was conducted by following the Dulmage (1970) [7] process. Each *B. thuringiensis* native isolate was grown for 72 h at 37 °C in 250 ml of LB broth. The pH of the culture broth of each isolate was reduced to 7.0 with 1 N HCl (from 8.4-8.7 in different isolates) and centrifuged for 10 minutes at 8000 rpm at 4 °C. The supernatant was discarded and the pellet was dissolved in 6.0 per cent lactose (1/10th volume of the initial broth). The suspension was stirred over a magnetic stirrer for 30 minutes and four volumes of cold acetone were added slowly, followed by another 30 minutes of stirring. The content was kept at 4 °C for another 2 hours to get complete precipitation of spores and crystal bodies and filtered through

Whatman No.1 filter paper under suction in a vacuum pump. The filtrate was discarded and the precipitate containing spores and crystals was allowed to dry in a vacuum desiccator at 25 °C overnight. The white crystalline powder obtained after drying was used to test bioefficacy against the larvae of *S. frugiperda*.

Insect rearing and bioassays

The *S. frugiperda* used in the present study was originally collected from Chikkaballapur during October 2018 and the iso-female colony was developed (NBAIR-MP-NOC-05a) and being maintained at Genomic Resources lab, ICAR-NBAIR, Bangalore. The adult moths were provided with 10% honey solution fortified with vitamin E. Larvae were reared on chickpea based semi synthetic artificial diet (Table 1).

All seventy *B. thuringiensis* isolates were tested at 100 ppm concentration. The crude protein of each isolate including reference strain HD1 were prepared by dissolving the calculated amount of crude protein in sterile distilled water. The toxicity of native *B. thuringiensis* isolates against the larvae of *S. frugiperda* was tested using diet incorporation method.

Approximately 1.0 ml of the toxin mixed diet (below 40 °C) was poured into each well of the bioassay tray (CD International trays™, Massachusetts, USA). A single healthy second instar larva was transferred to each well. A total of 60 larvae were tested under three replications. The bioassay trays were covered with self-adhesive pull-n-peel tabs (CD International pull-n-peel tabs™) and maintained in the environmental chamber (26 ± 1°C and RH 65 ± 5%, photoperiod of 16:8-L:D). The semi synthetic diet containing sterile water was maintained as untreated control. The number of dead larvae (larval mortality) was recorded at 3, 5 and 7 days after treatment. The mortality data were subjected to Abbott's correction. The corrected percent mortality was subjected to analysis of variance to find out the most effective *B. thuringiensis* isolates.

Results and Discussion

Screening of toxicity of *B. thuringiensis* isolates against the larvae of *S. frugiperda*

Preliminary screening of the *B. thuringiensis* native isolates was carried out against the freshly moulted second instar larvae of *S. frugiperda* and the findings are presented in Table 1. In preliminary screening assays, after 3 days after exposure, Bt-147 reported the highest mortality of 23.68 per cent among the native *B. thuringiensis* isolates, followed by Bt-272, Bt-18 and Bt-54 (21.50%) isolates, which are on par with each other. The Bt-31, Bt-41, Bt-106, Bt-248, Bt-258 and Bt-265 isolates were on par with each other with a mortality rate of 14.06 per cent. There was no mortality from Bt-83, Bt-90, Bt-92, Bt-110, Bt-201, Bt-202 and Bt-246 isolates (Table 1).

The larval mortality ranged from 4.25 and 36.84 per cent after 5 days of exposure. Bt-Oa1 reported the highest mortality of 36.84 per cent among the native *B. thuringiensis* isolates, followed by Bt-265 and Bt-Bt-31 (35.51%) isolates, which were on par with each other. The Bt-40, Bt-54, Bt-247 and Bt-275 isolates reported the next highest mortality of 34.21 per cent. The lowest mortality of 4.25 per cent was reported for the isolate Bt-92 (Table 1).

The larval mortality ranged from 13.16 to 86.84 per cent after 7 days of exposure. The Bt-Oa1 isolate reported the highest mortality of 86.84 per cent, but the reference strain HD-1 showed a maximum mortality of 89.47 per cent and was significantly superior to all the isolates tested. Isolates Bt-275,

Bt-265 and Bt-247 showed a mortality rate of 78.95 per cent, followed by isolates Bt-212 and Bt-142 (76.32%), which were equivalent to isolates Bt-21 and Bt-104 (73.68%). Isolate Bt-90 recorded the lowest mortality of 13.16 per cent.

To summarize, 50.0 per cent (35/70) of the *B. thuringiensis* isolates exhibited mortality in the range of 26-50 per cent while 10.0 per cent (7/70) of the *B. thuringiensis* isolates showed more than 75 per cent mortality after 7 days of exposure (Table 2).

The results of the current investigations are consistent with that of Valicente and Barreto (2003) [23] findings, who isolated 3408 *B. thuringiensis* strains from 1448 soil samples in 10 Brazilian states. Those strains have been tested against *S. frugiperda* larvae and only 12 per cent of *B. thuringiensis* strains showed mortality ranging from 78 to 100 per cent and did not cause mortality in 1758 isolates. The highest percentage of active strains (larval mortality above 75%) from the southern region was found.

Chilcot and Wigley (1993) [4] recorded that the percentage of soil isolates of *B. thuringiensis* had toxicity ranging from 37 to 88 per cent against lepidopteran larvae alone. In addition, Puntambekar *et al.* (1997) [19] tested various *B. thuringiensis* strains against certain lepidopteran pests and reported that 1018 spores per ml of *B. thuringiensis*.

All these results show a great difference among the isolates of the same subspecies of *B. thuringiensis* concerning their toxicity against *S. frugiperda*. This may be related to different protein genes of each strain in the same subspecies, which

results in various degrees of toxicity. Van Frankenhuyzen (2015) [24] pointed out that, besides the affinity of binding to the brush border membrane vesicles of the midgut of susceptible insect species, due to by diversity in toxicity spectra, other factors such as protoxin stability, differential solubilization of crystals and subsequent proteolytic processing are important, and emphasized that toxicity appears to be a function of the capacity of the toxin to form a pore in the membrane after binding to the receptor. Garcia *et al.* (2016) [8] pointed out that the gastric juice of *S. frugiperda* contains an inhibit factor, which decreases the pathogenicity of Bt. It must be pointed out that the surviving larvae in each treatment with Bt did not reach the fourth instar. According to Van frankenhuyzen (2015) [24], intoxication is associated with immediate feeding inhibition. This can be associated to the delaying of the larvae development and the reduction in the consumption, as observed by Lopes lastra *et al.* (1995) [13] for *S. frugiperda*, by Lambert *et al.* (1996) [12] for *S. littoralis* and by Regev *et al.* (1996) [20] for neonate *S. exigua* larvae. From the practical point of view, the control was complete, because the surviving larvae had their damage potential affected, not being able to cause injury to the crop. In the field, these weakened larvae could be easily killed by natural enemies. This situation shows a great advantage of the biological control compared to the chemical control, allowing for the contribution of the natural enemies to obtain a satisfactory control level of insect pests.

Table 1: Entomopathogenicity of *B. thuringiensis* isolates against 2nd instar larvae of fall armyworm, *S. frugiperda*

Sl. No	<i>B. thuringiensis</i> isolates	Corrected per cent mortality after		
		3 rd Day	5 th Day	7 th Day
1	Bt - 15	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	26.32 (30.86) ^f
2	Bt - 21	13.16 (21.27) ^b	28.95 (32.55) ^{bc}	73.68 (59.14) ^c
3	Bt - 23	5.26 (13.26) ^{de}	15.79 (23.41) ^{de}	23.68 (29.12) ^f
4	Bt - 26	2.63 (9.34) ^{ef}	13.16 (21.27) ^{de}	21.05 (27.31) ^f
5	Bt - 27	7.89 (16.32) ^{bc}	15.79 (23.41) ^{de}	28.95 (32.55) ^f
6	Bt - 28	7.89 (16.32) ^{bc}	10.53 (18.93) ^{de}	15.79 (23.41) ^g
7	Bt - 30	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	28.95 (32.55) ^f
8	Bt - 31	15.79 (23.41) ^{ef}	36.84 (37.37) ^a	68.42 (55.81) ^c
9	Bt - 34	5.26 (13.26) ^{de}	18.42 (25.42) ^c	34.21 (35.8) ^e
10	Bt - 35	2.63 (9.34) ^{ef}	15.79 (23.41) ^{de}	31.58 (34.19) ^f
11	Bt - 37	7.89 (16.32) ^{bc}	13.16 (21.27) ^{de}	28.95 (32.55) ^f
12	Bt - 38	13.16 (21.27) ^b	28.95 (32.55) ^{bc}	65.79 (54.2) ^d
13	Bt - 39	7.89 (16.32) ^{cde}	21.05 (27.31) ^{bc}	47.37 (43.49) ^e
14	Bt - 40	21.05 (27.31) ^a	34.21 (35.8) ^a	71.05 (57.45) ^c
15	Bt - 41	15.79 (23.41) ^b	26.32 (30.86) ^{bc}	55.26 (48.02) ^d
16	Bt - 43	13.16 (21.27) ^b	21.05 (27.31) ^{bc}	52.63 (46.51) ^d
17	Bt - 44	10.53 (18.93) ^b	15.79 (23.41) ^{de}	39.47 (38.92) ^e
18	Bt - 47	7.89 (16.32) ^{bc}	23.68 (29.12) ^{bc}	36.84 (37.37) ^e
19	Bt - 54	26.32 (30.86) ^a	34.21 (35.8) ^a	71.05 (57.45) ^c
20	Bt - 72	2.63 (9.34) ^{ef}	13.16 (21.27) ^{de}	31.58 (34.19) ^f
21	Bt - 83	0.01 (0.57) ^f	10.53 (18.93) ^{de}	26.32(30.86) ^e
22	Bt - 88	2.63 (9.34) ^{ef}	7.89 (16.32) ^e	21.05 (27.31) ^f
23	Bt - 90	0.01 (0.57) ^f	7.89 (16.32) ^e	13.16 (21.27) ^g
24	Bt - 92	0.01 (0.57) ^f	5.26 (13.26) ^f	21.05 (27.31) ^f
25	Bt - 103	5.26 (13.26) ^{de}	10.53 (18.93) ^{de}	26.32 (30.86) ^f
26	Bt - 104	13.16 (21.27) ^b	28.95 (32.55) ^{bc}	73.68 (59.14) ^c
27	Bt - 105	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	36.84 (37.37) ^e
28	Bt - 106	15.79 (23.41) ^b	26.32 (30.86) ^{bc}	68.42 (55.81) ^c
29	Bt - 107	10.53 (18.93) ^b	15.79 (23.41) ^{de}	39.47 (38.92) ^e
30	Bt - 108	7.89 (16.32) ^{bc}	18.42 (25.42) ^d	31.58 (34.19) ^f
31	Bt - 109	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	23.68 (29.12) ^f
32	Bt - 110	0.01 (0.57) ^f	7.89 (16.32) ^e	21.05 (27.31) ^f
33	Bt - 111	7.89 (16.32) ^{bc}	15.79 (23.41) ^{de}	36.84 (37.37) ^e
34	Bt - 113	13.16 (21.27) ^b	21.05 (27.31) ^{bc}	47.37 (43.49) ^e
35	Bt - 115	10.53 (18.93) ^b	18.42 (25.42) ^c	44.74 (41.98) ^e

36	Bt - 117	5.26 (13.26) ^{de}	23.68 (29.12) ^{bc}	42.11 (40.46)
37	Bt - 118	21.05 (27.31) ^a	26.32 (30.86) ^{bc}	57.89 (49.54) ^d
38	Bt - 122	5.26 (13.26) ^{bcd}	13.16 (21.27) ^{de}	34.21 (35.8) ^e
39	Bt - 126	7.89 (16.32) ^{bc}	21.05 (27.31) ^{bc}	42.11 (40.46) ^e
40	Bt - 129	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	26.32 (30.86) ^f
41	Bt - 132	7.89 (16.32) ^{bc}	10.53 (18.93) ^{de}	34.21 (35.8) ^e
42	Bt - 138	2.63 (9.34) ^{ef}	13.16 (21.27) ^{de}	28.95 (32.55) ^f
43	Bt - 139	5.26 (13.26) ^{bcd}	18.42 (25.42) ^d	39.47 (38.92) ^e
44	Bt - 142	13.16 (21.27) ^b	31.58 (34.19) ^b	76.32 (60.88) ^c
45	Bt - 146	7.89 (16.32) ^{bc}	15.79 (23.41) ^{de}	47.37 (43.49) ^e
46	Bt - 147	23.68 (29.12) ^a	34.21 (35.8) ^a	65.79 (54.2) ^d
47	Bt - 151	5.26 (13.26) ^{de}	15.79 (23.41) ^{de}	31.58 (34.19) ^f
48	Bt - 190	2.63 (9.34) ^{ef}	13.16 (21.27) ^{de}	28.95 (32.55) ^f
49	Bt - 201	0.01 (0.57) ^f	10.53 (18.93) ^{de}	36.84 (37.37) ^e
50	Bt - 202	0.01 (0.57) ^f	10.53 (18.93) ^{de}	34.21 (35.8) ^e
51	Bt - 212	7.89 (16.32) ^{bc}	23.68 (29.12) ^{bc}	76.32 (60.88) ^c
52	Bt - 216	10.53 (18.93) ^b	28.95 (32.55) ^{bc}	57.89 (49.54) ^d
53	Bt - 237	13.16 (21.27) ^b	15.79 (23.41) ^{de}	47.37 (43.49) ^e
54	Bt - 238	2.63 (9.34) ^{ef}	10.53 (18.93) ^{de}	36.84 (37.37) ^e
55	Bt - 240	7.89 (16.32) ^{bc}	15.79 (23.41) ^{de}	44.74 (41.98) ^e
56	Bt - 246	5.26 (13.26) ^{bcd}	10.53 (18.93) ^{de}	42.11 (40.46) ^e
57	Bt - 247	13.16 (21.27) ^b	34.21 (35.8) ^a	78.95 (62.69) ^c
58	Bt - 248	15.79 (23.41) ^b	31.58 (34.19) ^a	52.63 (46.51) ^d
59	Bt - 251	7.89 (16.32) ^{bc}	15.79 (23.41) ^{de}	55.26 (48.02) ^d
60	Bt - 256	10.53 (18.93) ^b	23.68 (29.12) ^{bc}	52.63 (46.51) ^d
61	Bt - 257	13.16 (21.27) ^b	26.32 (30.86) ^{bc}	86.84 (68.73) ^b
62	Bt - 258	15.79 (23.41) ^b	28.95 (32.55) ^{bc}	57.89 (49.54) ^d
63	Bt - 259	5.26(13.26) ^{de}	15.79 (23.41) ^{de}	44.74 (41.98) ^e
64	Bt - 265	15.79 (23.41) ^a	36.84 (37.37) ^a	78.95 (62.69) ^c
65	Bt - 267	10.53 (18.93) ^b	28.95 (32.55) ^{bc}	52.63 (46.51) ^d
66	Bt - 272	21.05 (27.31) ^a	31.58 (34.19) ^b	57.89 (49.54) ^d
67	Bt - 275	7.89 (16.32) ^{bc}	34.21 (35.8) ^a	78.95 (62.69) ^c
68	Bt - 278	7.89 (16.32) ^{bc}	26.32 (30.86) ^{bc}	55.26 (48.02) ^d
69	Bt - Oa1	18.42 (25.42) ^a	36.84 (37.37) ^a	86.84 (68.73) ^b
70	Btk HD-1	21.05 (27.31) ^a	39.47 (38.92) ^a	89.47 (71.07) ^a
	F-test	*	*	*
	SEm ±	3.99	3.64	2.24
	CD @ 1%	14.62	13.33	8.22

Values in the parentheses are arcsin transformed values. The values represented by same alphabet are statistically on par with each other by DMRT mean of three replications.

Table 2: Categorization of native *B. thuringiensis* strains according to their toxicity against 2nd instar larvae of *S. frugiperda*.

Sl No	Corrected percent mortality (7 th day)	No. of <i>B. thuringiensis</i> Isolates	% of <i>B. thuringiensis</i> isolates
1	0-25%	9.0	12.86
2	26-50%	35.0	50.00
3	51-75%	19.0	27.14
4	76-100%	7.0	10.0

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