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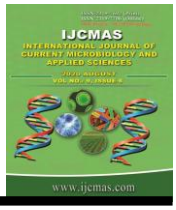

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Evaluation of Native Isolates of *Bacillus thuringiensis* against the Cotton Bollworm, *Helicoverpa armigera* (Hübner)

P. Swathi[1], S. Ramesh Babu[1]*, Devendra Jain[2], M.K. Mahla[1], Deepika Kalyan[1], P. Rokadia[3] and Poonam Yadav[4]

[1]Department of Entomology, [2]Department of Molecular Biology and Biotechnology, [4]Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology (MPUAT), Udaipur-313 001 (Rajasthan), India
[3]Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Banswara-327 001 (Rajasthan), India

**Corresponding author*

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A B S T R A C T

A total of twenty-eight native *Bacillus thuringiensis* strains were evaluated against neonate larvae of *Helicoverpa armigera* and found that three strains were effective with more than 80 % cumulative mortality, followed by seven strains with 51-75 and nine strains with more than 40% cumulative mortality at 7 days after treatment. Cumulative mortality of 83.3% was recorded in strain IBS1 which was statistically on par with each other and standard check *Bt. kurstaki* (HD-1) and also superior over other treatments. IBS5 and IBS14 also recorded higher cumulative mortality of neonate larvae of *Helicoverpa armigera*.

Introduction

Helicoverpa armigera (Hübner) (Lepidoptera; Noctuidae) is distributed from Europe, Africa, Asia and Australasia to the New World (Kriticos *et al*., 2015). In India, this pest is polyphagous in nature and present throughout the year and larval stage can survive and feed on 181 cultivated and wild plant species from about 45 families (Yogesh *et al*., 2016). Some of them are important agricultural crops like cotton, sorghum, groundnut, pigeonpea, chickpeas, tomato etc. Losses up to Rs.10, 000 million have been reported solely due to this pest in these crops (Indira, 2013 and Parmar *et al*., 2015; Chhangani, *et al.*, 2018).

Insecticide resistance and resurgence in different crops led to the indiscriminate use of insecticides against *H. armigera*. This pest shows resistance to all the major insecticide classes and it has become increasingly

difficult to control its population in India Indira, 2013. This has necessitated a search for more eco-friendly approaches to managing this pest (Reddy, *et al.*, 2019). Integrated Pest Management (IPM) is one of the reliable methods to achieve stabilityin crop production, as it uses all the appropriate techniques in a way that is as compatible as possible to keep the pest population below the economic threshold (Mathur and Kishor, 1987). Biological control is a crucial tool in IPM strategies. Biocontrol agents *viz*., predators, parasitoids, virus and microbes, play a key role in natural pest management activities, due to their specificity in attack, effectiveness, and safety to non-target organisms.

Bacteria belonging to the Bacillaceae family have a wide range of insecticidal activity, and from which *Bacillus thuringiensis* (*Bt*) is the most commonly used and successfully employed microbial pesticide in agriculture. The *B. thuringiensis*, which accounted for 90% of the world market of biopesticides, is pathogenic to nearly 525 species of insect-pests belonging to various orders (Khetan, 2001).

B. thuringiensis is a rod-shaped, gram-positive, spore forming ubiquitous aerobic typical soil bacterium, first discovered by Ishiwata in Japan, 1901 and then by Berliner, 1911 in Germany (Baum *et al., 1999). B. thuringiensis* produces a parasporal crystal protein, known as "Cry" protein (δ-endotoxin), which is responsible for producing insect pest toxicity (Crickmore et al., 2014). Due to their varied toxicity levels, different kinds of isolates have been studied and used in agriculture. Many insect pests belonging to Lepidoptera can be managed effectively with *Bt. Kurstaki* (Nethravathi *et al.*, 2010). Apart from major lepidopteran pests, *Bt* toxins are reported to show their toxicity on Diptera, Coleoptera, Orthoptera and also on nematodes, lice, mites, and aphids (Rosas-Garcia, 2009). Many countries have concentrated their research in biological control to isolate new local strains to use in pest management effectively. Accordingly, the present investigation aimed to isolate local *B. thuringiensis* for the control of *Helicoverpa armigera*.

Materials and Methods

Bt like isolates based on crystal morphology and twenty-eight Bt isolates were purified as single colony and maintained on specific medium (LB and T3 medium) and stored in glycerol stocks at -70_C for future studies.

Preparation of spore crystal mixture (Lenin *et al*., 2001)

The *Bt* culture was maintained in T3 agar plates at 4°C. Single colony of *Bt* was inoculated into 4 ml T3 broth and kept in a shaker, at 30°C temperature at 200 rpm for 48h. After overnight growth, the inoculum was added to 250 ml flask containing 25 ml T3 medium and incubated at 30°C on a rotary shaker maintained at 200 rpm for nearly 48 h. When more than 90 per cent lysis was reached, the culture was transferred to 4°C, at least half-an-hour before harvesting. The T3 broth containing spore-crystal mixture was centrifuged at 10,000 X g at 4 °C. The pellet obtained was resuspended in ice cold 1 ml Tris-EDTA buffer [Tris 10 mM, EDTA 1mM, pH 8.0 with 1mM phenylmethyl sulphonyl fluoride (PMSF)] and washed once with 1 ml ice-cold 0.5 M NaCl at 10, 000 X g for 10 min followed by two washes with 1 ml Tris-EDTA buffer at the same speed and time. Finally, the spore-crystal pellet was resuspended in 100 µl sterile distilled water with 1 mM PMSF and stored in -20°C.

Bioassay of local *Bacillus thuringiensis* isolates against *Helicoverpa armigera*

Mass screening of *B. thuringiensis* isolates

including commercial Bt (*B. thurigiensis kurstaki*HD-1) for toxicity against *H. armigera* was performed using relatively higher concentration (500 µg ml^{-1}) of spore-crystal mixtures. First instar larvae were used for bioassay by diet incorporation method (Song *et al*., 2003 and Lone *et al*., 2017). Thirty larvae per treatment were used. In the control, the culture was substituted with sterile distilled water. Mortality was recorded after 24 hrs to 7 days; larvae were scored as dead if they failed to respond to gentle probing. The experiment was set in triplicate, making total number of larvae tested per treatment to 72. The mortality observed was corrected to control mortality by Abbott's formula (Abbott, 1925). The data were subjected to ANOVA one way using minitab 19 online statistical package.

Results and Discussion

Inhibition of larval growth and toxic effects of twenty eight native isolates of *B. thuringiensis* against *Helicoverpa armigera* neonates after 1 day of treatment till 7 days was recorded. The reference strain exhibited the highest mortality rate of 9.72, 26.39, 26.38 and 23.61 at 1,3, 5 and 7 days after the treatment. Among all the isolates IBS 1 showed higher mortality rates of 9.72, 22.22, 30.55, 20.88 after 1,3, 5 and 7 days of exposure, respectively with a cumulative of 83.38 which was comparable with the standard strain. It was followed by IBS 14 with a similar mortality rate of 81.94. Isolates IBS 17, IBS28, IBS 42, and IBS 41 showed cumulative mortality rate of 64.15, 61.11 and 61.0, respectively. Isolates ISB32, ISB36, ISB19, ISB16 also showed promising toxic effects against *Helicoverpa* larvae with mortality rates of 56.93, 59.71, 50.38 and 54.15, respectively. They are followed by IBS3, IBS8, IBS9, IBS10, IBS18, IBS12 and IBS21 isolates with 16.66, 16.66, 19.44, 20.83, 16.88, 13.88 and 18.05 after 7 days of treatment (Table 1).

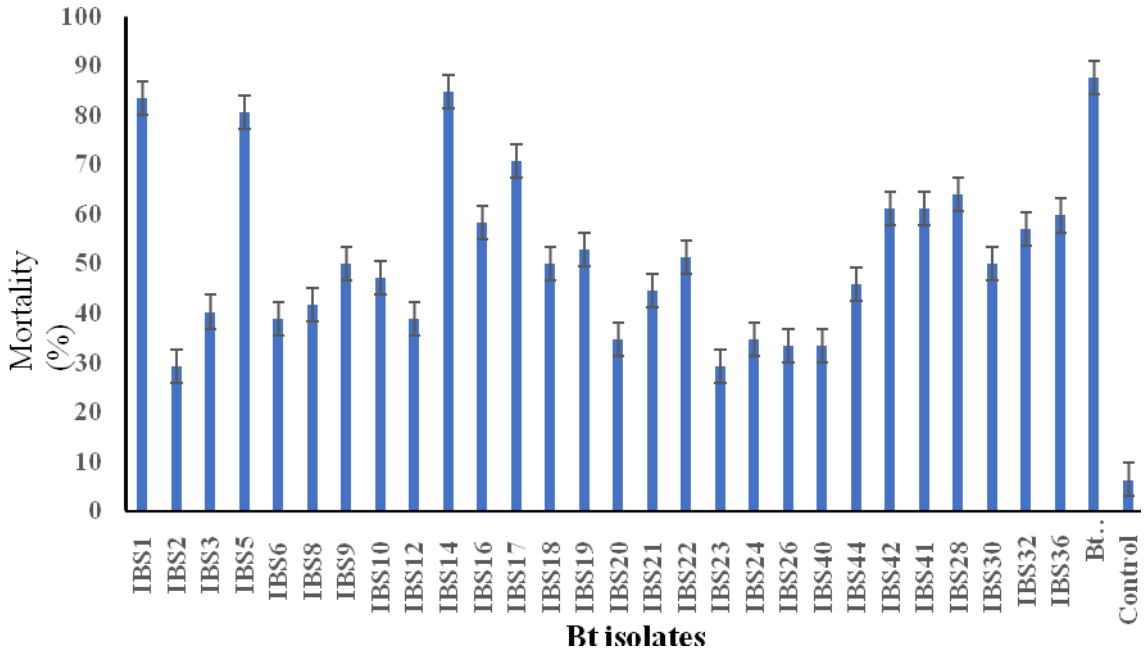
Table.1 Bioassay of native Bt isolates against neonate larvae of *H. armigera*

S. No.	Strain/Isolate	1st day	3rd day	5th day	7th day	Cumulative
1	IBS1	9.72 (18.06) a-e*	22.22 (28.08) ab	30.55 (33.56) a	20.88 (27.17)bcd	83.38 (65.98)ab
2	IBS2	1.39 (3.94) ef	4.16 (9.473) ij	8.66 (17.08)k-n	15.27 (22.92)ef	29.49 (32.85)m
3	IBS3	2.77 (7.89) def	5.55 (13.36) hij	15.27 (22.96)fgh	16.66 (24.04) def	40.26 (39.37)i-m
4	IBS5	15.27 (22.69) a	27.77 (31.80) a	19.44 (26.13)de	18.05 (24.98) cdef	80.54c (63.98)abc
5	IBS6	5.55 (11.16) b-f	9.72 (18.060) e-j	15.40 (23.06)fgh	8.33 (16.74)g	39.01 (38.58)i-m
6	IBS8	4.16 (11.83) c-f	8.66 (17.08) f-j	12.50 (20.70)h-k	16.66 (23.95) def	41.99 (40.36)h-m
7	IBS9	6.94 (15.10) a-f	9.72 (18.06) e-j	12.77 (20.93)g-j	19.44 (26.10) bcde	48.88 (44.35)e-l
8	IBS10	6.94 (15.10) a-f	8.33 (16.74) f-j	9.72 (15.14)j-m	20.83 (27.05)bcd	45.82 (42.58)f-m
9	IBS12	5.55 (13.47) b-f	9.72 (18.06) e-j	11.11 (19.45)i-l	13.88 (21.81)f	40.26 (39.36)i-m

10	IBS14	11.11 (19.38) a-d	26.39 (30.90) a	20.83 (27.15)cd	23.61 (29.04)ab	81.94 (64.95)ab
11	IBS16	4.16 (11.83) c-f	15.27 (22.92) b-f	12.50 (20.64)h-k	21.89 (28.01) bcde	54.15 (47.36)e-i
12	IBS17	12.50 (20.49) abc	18.05 (25.070) bcd	13.88 (21.86)g-i	26.39 (30.79) a	70.82 (57.47)bcd
13	IBS18	4.160 (11.83) c-f	16.66 (24.040) b-e	9.72 (18.14)j-m	16.88 (24.22)def	47.42 (43.53)f-l
14	IBS19	11.11 (19.38) a-d	8.55 (16.980) f-j	16.66 (24.04)efg	14.05 (21.95)f	50.38 (45.21)e-j
15	IBS20	4.16 (9.52) c-f	9.72 (18.06) e-j	6.27 (14.42)mn	13.88 (21.81)f	34.04 (35.62)j-m
16	IBS21	6.94 (12.48) a-f	8.22 (16.637) f-j	11.11 (19.44)i-l	18.05 (24.91) cdef	44.33 (41.690)g-m
17	IBS22	12.50 (20.70) abc	9.72 (18.06) e-j	13.89 (21.81)ghi	15.27 (22.63)ef	51.38 (45.79)e-i
18	IBS23	2.77 (7.89) def	6.93 (15.05) ghij	6.94 (15.22)mn	14.05 (21.81)f	30.53 (33.47)m
19	IBS24	4.16 (11.83) c-f	7.01 (15.12) g-j	8.33 (16.74)k-n	15.27 (22.92)ef	34.78 (36.07)j-m
20	IBS26	4.16 (9.52) c-f	9.72 (18.06) e-j	5.55 (13.54)mn	13.88 (21.81)f	33.32 (35.22)lm
21	IBS40	0.00 (0.00) f	9.94 (18.30) e-j	7.05 (15.17)mn	16.66 (23.95)def	33.66 (35.40)klm
22	IBS44	5.55 (13.47) b-f	13.89 (21.81) c-g	10.05 (18.42)i-m	15.27 (22.92)ef	44.76 (41.98)f-m
23	IBS42	9.72 (20.49) a-e	12.50 (20.49) d-h	15.29 (22.96)fgh	23.61 (28.89)ab	61.11 (51.41)def
24	IBS41	13.89 (21.81) ab	16.66 (23.95) b-e	16.66 (24.04)efg	13.88 (21.81)f	61.10 (51.47)def
25	IBS28	8.33 (16.42) a-f	20.83 (27.05) abc	18.05 (25.10)de	16.94 (24.27)def	64.15 (53.23)cde
26	IBS30	4.16 (9.52) c-f	12.50 (20.70) d-h	19.44 (26.12)de	14.05 (21.81)f	49.99 (44.97)e-k
27	IBS32	6.94 (15.10) a-f	11.11 (19.38) d-i	23.61 (29.06)bc	15.27 (22.92)ef	56.93 (48.97)d-h
28	IBS36	4.16 (9.52) c-f	13.89 (21.81) c-g	24.99 (29.98)b	16.66 (24.04)def	59.71 (50.59)d-g
29	*Bt kurstaki*	9.72 (18.06) a-e	26.39 (30.90) a	26.38 (30.87)b	23.61 (29.92)ab	87.49 (69.31)
a30	Control	0.00 (0.00) f	2.94 (9.75) j	1.39 (3.89)o	1.99 (8.04)h	6.32 (14.17)n

Figures in parentheses are arcsine transformed values; * Values followed by different letters (a, b, c, e, f, g, h, i, j) were significantly different (P<0.05, Tukey post-test)

Figure.1 Insecticidal Activity of Different Native Bt isolates against the First Instar Larvae of *Helicoverpa armigera*. Cumulative mortality was measured at the 7th day of assay. Mortality differed between different Bt isolates significantly and measured at P<0.05, Tukey test



Among the tenBt isolates, two were effective against *Spodoptera littoralis* and two more Bt isolates toxicity against *Helicoverpa armigera* (Merdan *et al*., 2010). Similarly, Patel *et al.*, (2018) isolated native Bt isolates and spore-crystal mixture of *B. thuringiensis* was tested at 10^7, 10^8 and 10^9concentrations with that of Btk HD-73 (standard check). Significant differences in all the screened Bt isolates were observed on the per cent mortality of *H. armigera*.

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