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Determination of LD_{50} and LT_{50} against *Spodoptera frugiperda* (J E Smith) for Native *Bacillus thuringiensis* Isolates

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ABSTRACT

Bacillus thuringiensis Berliner is a rod shaped, facultative, spore forming, gram positive and crystal bearing soil borne bacterium that is highly pathogenic and specific to insects. Several Strains of Bt were found effective against lepidopterans with highly infective strains like HD-1 which resulted in high larval mortality and was on par with chemical pesticides. To ascertain the insecticidal activity of six native *Bt* isolates *viz.,* isolate 49, 51, 52, 55 and 493, a bioassay was carried out on third instar larvae of *Spodoptera frugiperda* using a standard leaf dip method and efficacy was compared with a reference strain *B. thuringiensis* subsp. *Kurstaki* HD1. Mortality was calculated up to seven days after treatment. Efficacy was compared to Lethal dose (LD_{50}),which ranged between 2.64×10^7 (reference strain HD1) and 1.34×10^{11} CFU ml^{-1}(isolate 52)and LT_{50} in the range of 101.65 (reference strain HD1) to 146.16 hpi (isolate 52) at an uniform higher dose of 1×10^{12}.

Keywords: *Bacillus thuringiensis, hpi, Lethal dose, Lethal time and Spodoptera frugiperda*.

Maize with its highest genetic yield potential among all cereals is considered as "Queen of cereals". Due to its wider adaptability to different agro-climatic conditions, it is grown in 170 countries with an area of 193.7 m ha with an average production of 1147.7 million MT, productivity of 5.75 t/ha (FAOSTAT, 2020). In India, maize is grown in 9.63 m ha with annual production and productivity of 28.752 m t and 3065 kg ha^{-1}, respectively. In Andhra Pradesh,it is the 2nd most important cereal crop succeeding rice with an area of 0.336 m ha, annual production of 2.33 m t and productivity of 6912 kg ha^{-1}. Among all the districts of AP, Guntur ranks first with a productivity of 10,643 kg ha^{-1} (Ministry of Agriculture & Farmers Welfare, 2018-19).

The fall armyworm, *S. frugiperda* is a native insect pest to the tropical regions of the western hemisphere from the United States to Argentina. It is considered the most important pest of maize in Brazil, the third-largest producer of maize in the world after the USA and China. In India, fall armyworm was first reported in May 2018 as an invasive pest on maize fields of Shivamogga, Karnataka (Sharanabasappa*et al*., 2018). So far, it was reported from Karnataka, Telangana, Andhra Pradesh, Maharashtra, Tamil Nadu, and Gujarat states, causing severe foliar and cob damage to the maize crop. In Andhra Pradesh, fall armyworm incidence on maize was first noticed on 10th August 2018 at RARS, Anakapalle, Visakhapatanam district and later on, reported from different maize growing regions of Andhra Pradesh (Visalakshi*et al.*, 2019).

Various chemical insecticides are being promoted and used for FAW management, but

conditions where application practices are not feasible or the active ingredients are unsafe, there is a need to promote effective low risk bio pesticides. When used in conjunction with good crop management, these bio pesticides can help keep pest levels under control thus reducing the need to apply other insecticides (Bateman *et al.*, 2018). Microbial bio pesticides mainly include bacteria, fungi, viruses, protozoa and beneficial nematodes *etc*.Among the different microbial bio pesticides,*Bt*based bacterial bio pesticides comprise the major proportion.

MATERIAL AND METHODS

The experiment was conducted at Department of Entomology, Agricultural College, Bapatla. Five native lepidopteran specific *B. thuringiensis* isolates that were present in the repository of insect pathology lab at Agricultural College, Bapatla were used in the present study. All the *Bt*isolates were multiplied by inoculating into T_3 broth. The number of colonies in the broth was counted by serial dilution technique. Different concentrations of each of the native *Bt* isolate (49, 51, 52, 55 and 493) and reference strain HD 1 were prepared from 10^{-1} to 10^{-10} concentrations. From each dilution, 100 µl of aliquot was taken and spread on Petri plates containing T_3 medium with spreader and incubated at 34-37 ^{0}C for 24 h. Colony counting was done after 24 h based on the standard formula.

$$\text{Number of colonies per milliliter of sample} = \frac{\text{Number of colonies}}{\text{The aliquot taken x dilution factor}}$$

To ascertain the insecticidal activity of native *B. thuringiensis* isolates, a leaf dip bioassay experiment (Shelton *et al.*, 1993) was carried out along with reference strain *B. thuringiensis* sub sp. *Kurstaki* (HD 1). Tender maize leaves were cut into uniform size and were dipped in *B. thringiensis*T_3 broth which is at a concentration of 1×10^{12} CFU ml^{-1} which contained 0.1 per cent Tween 80 solution and air-dried. Later, the treated leaves were placed in plastic rearing cups and one larva per cup was released. Twenty third instar larvae were kept for each replication. Three replications were maintained one in each tray along with leaf dipped in distilled water as a control. Larvae were transferred to fresh leaf diet after 24 hours. Larval mortality was recorded at every 24 hours up to seven days after treatment. The moribund larvae were also considered as dead (Plate 1). The mortality in control was also recorded and corrected mortality (Abbott, 1925) per each treatment was calculated.

$$\text{Corrected larval mortality (\%)} = \frac{(\%\text{ Mortality in treatment}-\%\text{ Mortality in control}}{(100-\%\text{Mortality in control})} \times 100$$

The relative toxicity of native *Bt*isolates was calculated by taking isolate-52 as a standard.

$$\text{Relative toxicity} = \frac{\text{LC50 of native } Bt \text{ isolate 52}}{\text{LC50 of } Bt \text{ isolate}}$$

Statistical analysis

The mortality data generated from the bioassay study were subjected to probit analysis for calculation LD_{50} and LT_{50}values (Finney, 1984) using Statistical Package for Social Science (SPSS) ver.20.0 software.

RESULTS AND DISCUSSION

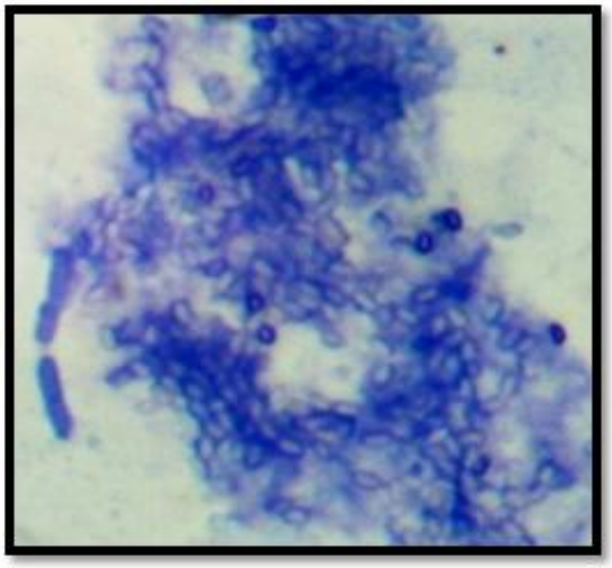
Lethal Dose (LD_{50})

Native *Bt* isolates showed LD_{50} (table.1, Fig. 1.) in the range of 1.34×10^{11} to 2.64×10^{7} CFU ml^{-1}. The reference strain HD1 recorded the lowest median lethal dose of 2.64×10^{7} CFU ml^{-1}. Among the native *Bt*isolates the lowest LD_{50} value was recorded by the isolate-493 with 2.03×10^{8} CFU ml^{-1} followed by isolate-51, isolate-55 and isolate-49 with LD_{50} values

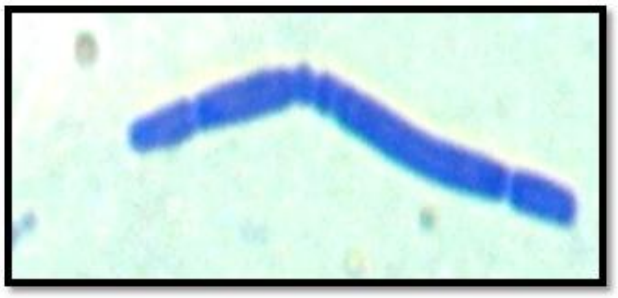
Larvae kept for pre starvation

Maize leaves treated with *Bt*isolates

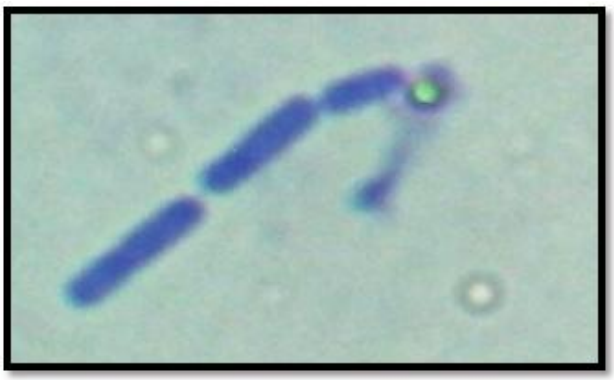
Dead larvae infested with *Bt*



Crystal staining



Staining to confirm the presence of *Bt*in dead larvae



Endospores staining

Plate 1. Bioassay to test the efficacy of native *Bt* isolates on *S. frugiperda*

Table 1. Median lethal dose mortality response of third instar larvae of *S. frugiperda* with native isolates of *Bt*

Isolate	Chi-square (χ^2)	Regression equation	*LD50 (CFU ml^{-1})	Fiducial limit (CFU ml^{-1})
I-HD1	0.064	Y= -2.214 + 0.298 X	2.64×10^7	(1.98×10^6)-(1.18×10^8)
I-49	0.071	Y= - 2.180 + 0.203X	5.38×10^{10}	(1.51×10^{10})-(3.44×10^{11})
I-51	0.209	Y= - 2.557 + 0.251X	1.51×10^{10}	(5.26×10^9)-(4.71×10^{10})
I-52	0.399	Y= - 2.352 + 0.211X	1.34×10^{11}	(3.64×10^{10})-(1.12×10^{12})
I-55	0.658	Y= - 2.212 + 0.207X	4.71×10^{10}	(1.35×10^{10})-(2.74×10^{11})
I-493	0.591	Y = - 2.449 + 0.295 X	2.03×10^8	(3.45×10^7)- (6.34×10^8)

* Mean of three replications (N=20)

Table 2. Median lethal time mortality response of third instar larvae of *S. frugiperda* with native isolates of *Bt*

Isolate	Chi-square (χ^2)	Regression equation	LT50 (hpi) at 1×10^{12} CFU ml^{-1}	Fiducial limit (hpi)
I-HD1	2.233	Y= - 13.545 + 6.748 X	101.65	97.20 – 106.07
I-49	9.907	Y= - 13.470 + 6.279 X	139.73	133.31 – 147.53
I-51	10.085	Y= - 14.301 + 6.734 X	133	127.41 – 139.37
I-52	7.09	Y= - 13.235 + 6.114 X	146.16	138.95 – 155.43
I-55	5.909	Y= - 13.967 + 6.477 X	143.32	136.74 – 151.52
I-493	1.504	Y= - 13.346 + 6.529 X	110.71	105.93 – 115.60

Table 3. Relative toxicity of *Bt* isolates against *S. frugiperda* 3rd instar larvae after treatment

Isolate	Relative toxicity (LD_{50})	Relative toxicity (LT_{50})	Order of toxicity	
			LC_{50}	LT_{50}
I-HD1	5.07×10^3	1.44	1	1
I-49	2.5	1.04	5	4
I-51	8.87	1.09	3	3
I-52	1	1	6	6
I-55	2.85	1.01	4	5
I-493	6.60×10^2	1.32	2	2

of 1.51×10^{10}, 4.71×10^{10} and 5.38×10^{10} respectively. The highest LD_{50} value was obtained with the isolate-52 (1.34×10^{11} CFU ml^{-1}) and thus found to be the least effective out of all the native isolates tested.

The Chi-square values (c^2), fiducial limits and regression equation corresponding to the tested *Bt* isolates were presented in the table. The regression equation indicated an increase in mortality with an increase in concentration. The c^2 values showed that the test population was homogenous with a goodness of fit. A lethal dose response plotted against the probit mortality was shown in the fig 1.

Lethal Time (LT_{50})

The LT_{50} values of different native *B. thuringiensis* isolates were calculated for a uniform higher dose of 1×10^{12} CFU ml^{-1} against third instar larvae of *S. frugiperda* was shown in Table. 2. Lethal time response was plotted against probit mortality was shown in the Fig.2.

Out of all the *Bt*isolates tested the reference *Bt* strain HD1 achieved the fastest kill with 50 per cent mortality at 101.65 hours. Native *Bt*isolates besides the reference strain showed the LT_{50} values in the range of 110.71 to 146.16 hours. Of all the native *Bt*isolates, the isolate 493 has achieved the fastest lethal action on the third instar larvae of *S. frugiperda*with 110.71 hpi, followed by isolates 51, 49 and 55 with the lethal time for 50 percent mortality as 133.00, 139.73 and 143.32 hpi, respectively. The slowest kill was observed with the isolate 52 (146.16 hours).

Relative toxicity

The descending order of relative toxicity of test insecticides based on LC_{50} values was isolate HD1 (5.07×10^{3}) > isolate 493 (6.60×10^{2}) > isolate 51 (8.87) > isolate 55 (2.85) > isolate 49 (2.50) > isolate 52 (Table 3.)

Similarly, based on LT_{50} values the relative toxicity of isolate HD1 (1.44) > isolate 493 (1.32) > isolate 51 (1.09) > isolate 49 (1.04) > isolate 55 (1.01) > isolate 52 (Table 3.)

Molecular characterisation of isolates 49, 51, 52, 55 (same isolates used in the study) by Pavani (2019) reported the presence of *vip*3, *cry* 2, *cry* 9 and *cry* 9 genes, respectively. The reference strain HD1 was reported for the presence of *cry* 1 gene. The fastest lethal action of reference strain was due to higher susceptibility of *S. frugiperda* larvae to *cry* 1 gene. Similarly, Murali Krishna *et al.* (2018) reported that lethal times of four isolates, F493, F468, N30 and N115 were 78.52, 74.28, 95.70 and 88.68 hours, respectively against *S. litura*.

The results were in line with work done by Da Silva *et al.* (2004) who tested the efficacy of native *B. thuringiensis* isolates S701, S764 and S1265 against second instar larvae of fall armyworm and reported the LC_{50} values of 6.512×10^{-7}, 2.188×10^{-6}, 4.081×10^{-5} dilutions of the culture respectively. Isolate HD1 recorded the LC_{50} value of 1.706×10^{-6} dilutions. Similarly, Polanczyk*et al.* (2000) tested various *Bt*isolates (dendrolimusHD 37,aizawaiHD 68, kurstakiHD 73, darmstadiensisHD 146, and4412) against second instarlarvae of *S. frugiperda*at different concentrations 8×10^{5}, 3×10^{6}, 8×10^{7}, 3×10^{8} cells ml^{-1} and reported *Bt*aizawaiHD 68 as most effective strain with an LC_{50} of 6.7×10^{6} cells ml^{-1} due to the presence of two genes *cry*IA, *cry*ID related to toxicity and their interaction with each other and *Bt*4412 was next effective with 8.6×10^{6} cells ml^{-1}.

SUMMARY AND CONCLUSION

The median lethal dose (LD_{50}) ranged from 2.64×10^{7} to 1.34×10^{11} CFU ml^{-1}. The lowest LD_{50} valuewas recorded with reference stain HD 1 (2.64×10^{7} CFU ml^{-1}) and the highest LD_{50} was recorded for isolate-52 (1.34×10^{11} CFU ml^{-1}). Among the native *Bt* isolates the fastest lethal action against *S. frugiperda*larva was shown by HD1 (101.65 hpi) followed by isolate-493, 51, 49, 55. The highest LT_{50} was recorded by isolate 52 (143.32 hpi).

The fastest lethal action against *S. frugiperda*larva was shown by reference *Bt*strain HD1 (101.65 hpi) followed by isolate 493 (110.71 hpi), 51 (133.00 hpi), 49 (139.73 hpi) and 55 (143.32 hpi). The highest LT_{50} was recorded by isolate 52 (143.32 hpi).

From the present investigation, it was concluded that fastest kill with respect to LD_{50} and

LT_{50} on *S. frugiperda* was achieved in the descending order of *cry* 1 >*cry* 2 >*vip*3 >*cry* 9 genes.

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