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EFFICACY OF NATIVE ISOLATES OF *Bacillus thuringiensis* Berliner ON MORTALITY OF 3rd INSTAR LARVAE OF FALL ARMY WORM, *Spodoptera frugiperda* (J.E. Smith)

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ABSTRACT

Four native isolates of *Bacillus thuringiensis* Berliner *viz.*, V13, CF24, CFo76 and W85 (collected from soils from in and around different locations from Tirupati, Chittoor Dt., Andhra Pradesh, India) were tested at five different concentrations against third instar larvae of Fall Army Worm *Spodoptera frugiperda* (J.E. Smith). Among the four native isolates tested, V13 isolate showed highest per cent larval mortality (86.66%), Lowest values of LC_{50} (6.28×10^2 CFU/ml) and LT_{50} (69.02 h) for third instar larvae of FAW, indicating that the isolate V13 could be used as a potent *Bt* native isolate for the management of third instar larvae of *S. frugiperda*.

KEYWORDS: *Bacillus thuringiensis*, LC_{50}, LT_{50}, Native isolates, per cent larval mortality, *Spodoptera frugiperda*

INTRODUCTION

Maize is the third most important cereal crop after rice and wheat in India, sharing about 2 per cent the world's maize production. About 71 per cent of maize in India is being produced during the *kharif* season in different states *viz.,* Karnataka, Madhya Pradesh, Tamil Nadu, Maharashtra, Telangana, Uttar Pradesh and Rajasthan with Karnataka being the leader. Bihar, Andhra Pradesh and Tamil Nadu are the states in India that accounts to 40 per cent of maize crop in *rabi* season (Anonymous, 2017).

Among the biotic constraints that limit the maize production, insect pests *viz.,* stem fly, stem borer, cornworm, aphids, shoot fly *etc.* are the major insect pests inflicting maximum yield loss. Fall ArmyWorm, *Spodoptera frugiperda* popularly known as FAW is native to tropical and subtropical Americas. FAW is a polyphagous pest which majorly prefers plants/crops belonging to Poaceae family and most commonly recorded in wild and cultivated grasses like maize, rice, sorghum and sugarcane (Anonymous, 2020). It has been reported that around 353 host plant species of 76 plant families *viz.,* Poaceae (106), Asteraceae (31) and Fabaceae (31) are preferred as host by FAW (Montezano *et al.,* 2018).

Invasion and existence of *S. frugiperda* in India was confirmed by the University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka during May-June, 2018 and since then this insect has become a major threat to maize cultivation in India (Sharanabasappa *et al*., 2018). It has been reported that damage by this insect pest causes a three per cent reduction in grain yield (Lima *et al*., 2010) and with an annual loss up to US dollars 400 million in Brazil (Figueiredo *et al.,* 2015). During the year, 2018 due to this pest in India, production of maize fell by 3.2 per cent equivalent to 27.8 million tons of grain.

Farmers rely predominately on the use of synthetic insecticides for managing this insect pest. Use of insecticides as a sole tool in management of insect pests has potential draw backs, that includes effect on the environment, non-target organisms and natural enemies coupled with outbreak of secondary pest and resurgence (Togbe *et al.,* 2014). An attractive alternative tool for management of insect pests is the use of biological pesticides due to their ecofriendly and target selective characteristics (Ali *et al.,* 2015) and microbial agent containing insecticides are considered as good replacement due to the absence of mammalian toxicity (Sabbour, 2003). Biopesticides like *B.t, and Beauveria bassiana* can provide an alternative and environment friendly option to control several important insect pests (Taggar *et al.,*2014).

Over 100 bacteria have been identified as insect pathogens, among them, *B.t.* has got maximum importance

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as a microbial agent (Muhammad *et al.,* 2016). *B.t* is a gram positive, spore forming bacterium having insecticidal properties and is benign to natural enemies, quite safe to mammals and also environmentally acceptable (Ali *et al.,* 2015). *B.t* produces crystal toxins that activates into protoxins in the insect gut due to alkaline pH of 9.0-9.5. due to insertion of protoxins on receptor proteins of midgut membrane, results in formation of pores in the gut resulting gut paralysis and septicemia causing the death of larvae.

MATERIAL AND METHODS

The research work was carried out at Insectary and Insect pathology laboratory, Department of Entomology, S.V. Agricultural College, Tirupati during 2020 –21.

Rearing and maintenance of *S. frugiperda*

Rearing of *S. frugiperda* was done at the Insectary, Department of Entomology, S.V. Agricultural College, Tirupati at temperature of 25 ± 2°C, relative humidity of 75.00 ± 5.00 per cent and photoperiod of 12 h light / 12h dark. Eggs of *S. frugiperda* were collected from surrounding farmer's maize fields and were kept in plastic troughs (200mm diameter and 100 mm height).First instar larvae were reared in groups on a corn flour based artificial diet, late instars were reared individually up to pupation (Barreto *et al.*,1999).

Larvae took 15-20 days to complete the larval duration and pupated in rearing troughs. The pupae were collected and were shifted to adult rearing cages (35 × 25 × 45 cm) provided with a maize seedling as oviposition substrate. A cotton swab dipped in 10 per cent honey solution was provided as food material for the emerging adults. Eggs laid by the adults on the maize seedlings were collected and the hatched larvae were reared on the artificial diet up to third instar (identified by change of instar, width of head capsule *i.e.,* 0.81 to 0.95 mm and duration (4 to 6 days from hatching) and these third instar larvae were used for bioassay studies.

Culturing of *B.t.*

Forty ml of Luria Bertani broth was taken in 50ml conical flask and sterilized in autoclave at 121°C, 15 lbs pressure for 15 minutes. After cooling, the broth was inoculated with one loop of each native *B.t* isolate in different conical flasks. The flasks with culture broth were subjected to shaking for 48 h. Serial dilutions were prepared at 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} dilutions and 100 µl of each dilution was taken and spread on Luria agar plate with 'L shaped' rod. The plates were kept in incubator for overnight at 37°C. After 24 h colony count was taken based on the standard formula. (Aneja, 2003).

The number of bacteria colony or CFU /ml =

$$\frac{\text{No. of colonies}}{\text{Volume taken} \times \text{Dilution factor}}$$

After colony counting, a standard concentration of 1.5×10^7 CFU/ml was prepared for all the isolates from which serial dilutions *viz.,* 1.5×10^7; 10^6; 10^5; 10^4; 10^3 CFU/ml were made and used for the bioassay experiments.

Bioassay studies of *B.t.* against third instar larvae of *S. frugiperda*

Five grams artificial diet cubes were dipped in different dilutions of native *B. t.* isolates for 4 to 5 min then air dried. Ten third instar larvae of *S. frugiperda* were released on to the artificial diet and were allowed to feed on treated artificial diets. The experiment was replicated three times. Thirty larvae were tested for each dilution and a total of five concentrations were used for each isolate. Commercial formulation (Dipel) was also prepared into different concentrations and used as check. The diet treated with distilled water was served as control. Larval mortality was recorded after 24 h of treatment at regular intervals and continued to till pupation or death of the larvae. Per cent larval mortality was recorded by dividing number of larvae died out of total number of larvae treated.

Statistical analysis

The recorded larval mortality was converted into percentage values by using the following formulae and then transformed to arc-sine values. Mean values were separated by DMRT.

Per cent larval mortality =

$$\frac{\text{No. of larvae died due to infection}}{\text{Total no. of larvae treated}} \times 100$$

The larval mortality percentage data obtained from *B.t* bioassay, were subjected to Probit analysis for determining LC25, LC50, LC75 and LC99 values with

the help of SPSS package.

RESULTS AND DISCUSSION

Among native isolates and commercial formulation tested, the mean larval per cent mortality recorded at different concentrations were presented in Table1.

Among the native isolates tested (at 1.5×10^7 CFU/ml) the highest mortality was observed in V13 isolate (86.66%) followed by W85 isolate (73.33%) which were significantly different from each other. The larval mortality of V13 isolate (90.00%) was on par with Dipel (commercial formulation), whereas no mortality was recorded in the control treatment.

The present investigation revealed that the mortality has increased with an increasing in the concentration indicating a positive correlation between the mortality of the larvae and the dose applied. In accordance to the present study, Pavani (2019) evaluated 15 native *B.t*. isolates against third instar larvae of *Spodoptera litura* at different concentration and found that the mean percent larval mortality (which were in the range of 33.33 to 90.00%) was dose dependent.

In the present investigation though all the native isolates tested showed promising results (around 80.00% mortality against third instar larvae of *S. frugiperda*) the commercial formulation Dipel had shown highest mortality (90.00%) surpassing the native isolates, this probably is due to role of strain in the commercial formulation Dipel *i.e., B.t kurstaki.*

Nethravathi and Huger (2010) also investigated the efficacy of *B.t* isolates against five-day-old larvae of cabbage leaf webber and diamond back moth and found that the standard controls; Dipel and HD1, had the highest mortality rates (90.00 and 80.00% against leaf webber, respectively), compared to the native isolates 2422c (80.00%). Similarly, for *Plutella xylostella*, the mortality rates with the reference strains were 99.97 and 86.67 per cent which can comparable to native strain 2458c with 80.00 per cent mortality.

Similarly, Lalitha and Muralikrishna (2012) reported a mortality range of 10 to 93.33 per cent among the 114 native *B.t* isolates against first and third instar *S. litura* larvae. While the highest mortality was recorded in the

Table 1. Mean per cent mortality of third instar larvae of *S. frugiperda* at different concentrations of native *B.t.* isolates

Concentrations CFU/ml	Isolates				
	V13	CFo76	W 85	CF24	Dipel
10^3	30.00^{klm}	13.33^{o}	23.33^{mn}	16.67^{no}	36.67^{ijk}
	(33.21)	(21.14)	(28.78)	(23.85)	(37.22)
10^4	36.67^{ijk}	26.67^{lmn}	33.33^{jkl}	30.00^{klm}	50.00^{efg}
	(37.22)	(30.99)	(35.22)	(33.21)	(45.00)
10^5	43.33^{ghi}	40.00^{hij}	43.33^{ghi}	43.33^{ghi}	63.33^{c}
	(41.15)	(39.23)	(41.15)	(41.15)	(52.77)
10^6	63.33^{c}	46.67^{fgh}	56.67^{cde}	53.33^{def}	76.67^{b}
	(52.75)	(43.07)	(48.84)	(46.92)	(61.21)
10^7	86.66^{a}	60.00^{cd}	73.33^{b}	63.33^{c}	90.00^{a}
	(68.83)	(50.77)	(59.00)	(52.77)	(71.57)
Isolate means	43.33^{b}	31.11^{c}	38.33^{c}	34.44^{d}	52.79
	(38.85)	(30.86)	(35.49)	(32.97)	(44.61)
Control			0.00%		
	Isolates	**Concentrations**		**Isolate × concentration**	
Sem±	1.083	1.186		2.653	
CD	3.071	3.364		7.523	

* : Values in the parenthesis are angular transformed values and mean values followed by same alphabet in column are not significantly different

Table 2. Lethal concentration (LC_{25}, LC_{50}, LC_{75} and LC_{99}) values for native *B.t* isolates against third instar larvae of *S. frugiperda*

Lethal concentration	V13	CFo76	W85	CF24	DIPEL
LC25	6.28×10^2	1.02×10^4	1.95×10^3	4.4×10^3	1.79×10^2
Fiducial limits	2.24×10^1 to 3.36×10^3	3.88×10^2 to 5.12×10^4	3.68×10^1 to 1.17×10^4	1.03×10^2 to 2.45×10^4	0.21×10^1 to 1.36×10^3
LC50	2.64×10^4	1.31×10^6	2.16×10^5	5.78×10^6	9.68×10^3
Fiducial limits	5.66×10^3 to 8.71×10^4	2.76×10^5 to 2.42×10^7	4.78×10^4 to 1.40×10^6	1.27×10^5 to 6.46×10^6	1.23×10^3 to 3.55×10^4
LC75	1.11×10^6	1.69×10^8	2.39×10^7	7.59×10^7	5.24×10^5
Fiducial limits	2.98×10^5 to 1.08×10^7	1.21×10^7 to 1.86×10^{15}	2.93×10^6 to 3.56×10^9	6.70×10^6 to 3.96×10^{10}	1.39×10^5 to 4.85×10^6
LC99	1.05×10^{10}	2.48×10^{16}	2.44×10^{15}	2.32×10^{16}	9.24×10^9
Fiducial limits	3.25×10^8 to 3.00×10^{15}	4.44×10^{10} to 1.66×10^{24}	1.02×10^{10} to 5.33×10^{21}	2.69×10^{10} to 3.10×10^{23}	2.37×10^8 to 5.11×10^{16}
Regression equation	Y= -1.84 + 0.41X	Y= -1.96 + 0.32X	Y = -1.76 + 0.33X	Y = -1.84 + 0.32X	Y = - 1.02 + 0.32X
Chi-square	0.917	0.461	0.231	0.286	0.576

* Y = a + bx; where Y = Probit; X = Concentration (CFU ml^{-1}); a = intercept; b = slope

reference strain, HD1(93.33 and 76.67 per cent in first and third instar larvae, respectively).

Thilagavathi *et al*. (2020) reported the toxicity of four *B.t.* isolates against third instar larvae of *P. xylostella* and the isolate CC recorded the maximum mortality of 95.33 per cent comparable to the standard check HD1 98.31 per cent.

Determination of Lethal Concentration (LC) of *B.t* isolates against *S. frugiperda* (Table 2)

For all the four native isolates of *B.t* tested; LC25 values ranged from 6.28×10^2 to 1.02×10^4 CFU/ml. The lowest LC25 value was recorded in V13 (6.28×10^2 CFU/ml) followed by W85 with 1.95×10^3 CFU/ml. whereas the highest value was recorded in the isolate CFo 76 with 1.02×10^4CFU/ml

The LC50 values for the four native isolates tested were in the range from 2.64×10^4 to 5.78×10^6 CFU/ml. The lowest LC50 was recorded in V13 isolate (2.64×10^4 CFU/ml) followed by W85 isolate (2.16×10^5CFU/ml), while the highest LC50value was noted in the isolate CF24.

The LC75 for the four native isolates tested ranged from 1.11×10^6 to 1.69×10^8 CFU/ml, with the lowest LC75 in V13 (1.11×10^6CFU/ml) followed by W85 2.39×10^7 CFU/ml. The highest LC75 value was recorded in the isolate CF24 (7.59×10^7CFU/ml).

LC99 values of the four native isolates ranged from 1.05×10^{10} to 2.48×10^{16} CFU/ml. The lowest LC99 value was observed in V13 (1.05×10^{10}CFU/ml.) followed by W85 with 2.44×10^{15}CFU/ml. Meanwhile the highest LC99 value was recorded in CFo76 with 2.48×10^{16} CFU/ml while for the CF24 was 2.32×10^{16}CFU/ml. which was similar to the CFo76 isolate. Similar to the per cent larval mortality from earlier bioassay results (Table 1) the lowest LC25, LC50, LC75 and LC99 was observed in commercial Dipel (Table 2) indicating its highest efficacy.

Table 3. Lethal time (LT) values for native *B.t* isolates against third instar larvae of *S. frugiperda*

Strain	Regression equation	X^2	LT_{50}	Lower limit	Upper limit	LT_{90}	Lower limit	Upper limit
V13	Y = -4.98 + 2.71X	1.27	69.02	56.97	81.91	205.03	154.85	337.17
CFo76	Y = -5.06 + 2.43X	0.77	121.19	99.08	167.80	408.44	255.99	1130.94
W85	Y = -4.88 + 2.57X	0.22	78.54	65.17	94.81	247.26	178.45	455.04
CF24	Y = -5.11 + 2.54X	0.45	103.65	86.37	132.75	331.81	222.78	746.67
Dipel	Y = -4.15 + 2.38X	1.47	54.72	42.47	66.36	188.55	139.73	325.43

* Y = a + bx; where Y = Probit; X = time; a = intercept; b = slope

However, among the native isolates tested the lowest LC25, LC50, LC75 and LC99was observed in the isolate V13 ranking it as most effective isolate. Valicente and Lana (2008) conducted bioassay studies of *S. frugiperda* with *B. t* and determined the LC50 by using doses ranging from 10^3 to 10^9 spores /ml against two-day old healthy fall army worm larvae. LC50 was 8.21×10^6 spores /ml for strain 344 while strain 1644 showed LC50 of 2.07×10^6 spores /ml.

Murali Krishna *et al.* (2018) recorded the least lethal concentration in HD1 strain of *B.t.* as 9.56×10^4 CFU/ml followed by 9.76×10^4 CFU/ml with F493 isolate and 1.90×10^5 CFU/ml with N30 isolate when screened against third instar larvae of *S. litura.*

Determination of Lethal Time (LT) of *B.t* isolates against *S. frugiperda*

Among the four native isolates tested, the least LT_{50} value was recorded in the V13 isolate with 69.02 hours followed by W85 with 78.54 hours. Whereas for the commercial check, Dipel the LT_{50} value was 54.72 hours. Regression equation, fiducial limits and chi square values were presented in Table 3.

Prabagaran *et al.* (2002) reported that LT values of live *B.t.* strains, *viz.,* PBT-782, PBT 372, PBT-574, PBT801 and PBT-716 were 25.46, 36.81, 48.18, 50.35 and 73.53 h, respectively.

Similar to the present investigation, Murali Krishna *et al.* (2018), reported that four isolates (E468, E493, N30, N115) among 21 *B.t.* isolates were found to be potent isolates with a median lethal time of 74.28 h 78.52 h, 88.68 h, and 95.70 h, respectively.

Among the four native isolates tested, V13 isolate showed the highest per cent larval mortality (86.66%), Lowest values of LC50 (6.28×10^2 CFU/ml) and LT50 (69.02 h) indicating that the isolate V13 could be the most potential *B.t* native isolate for the management *S. frugiperda.*

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