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Determination of LT_{50} against *Spodoptera litura* (Fab.) for Native *Bacillus thuringiensis* (Berliner) Isolates from Soil Samples in Guntur District of Andhra Pradesh

Keywords: *Bacillus thuringiensis,Spodoptera litura*, LT_{50}

The tobacco caterpillar, *Spodoptera litura* (Fab.) is one of the polyphagouspest throughout world infesting 112 species of plants belonging to 44 families, of which 40 species are known from India (Chari and Patel, 1983). Indiscriminate use of insecticides to control the pest leads to resistance development, resurgence and deleterious effects to environment and non-target organism. The stock of *S. litura*had developed high level of resistance to pyrethroids and moderate to high level of resistance to organophosphate (Saleem *et al*., 2015). In order to minimize the toxic effects of insecticides, search for non insecticidal methods were initiated anddevelopment of natural biocontrol agents is the best alternative to control of *S. litura*. This method was found effective to manage insects by the usage of entomopathogenic bacteria. Among entomopathogenic bacteria, *Bacillus thuringiensis*(Berliner) was reported toxic to Lepidopteran, Dipteran and Coleopteran insect pests (Schnepf*et al*., 1998). In this scenario, Keeping importance of *B. thuringiensis*in view, soil samples were collected from Guntur district to isolate *B. thuringiensis*.

The present investigation was carried out at Department of Entomology, Agricultural College, Bapatla during 2018-19. A total of 62 soil samples were collected during pre-sowing and mid crop season (45-50 days) of the cotton from five mandals of Guntur district. Samples were stored at 4 R"C for further processing. Samples were isolated by sodium acetate selection method suggested by Travers *et al*. (1987). Samples were buffered with 0.25 M sodium acetate where undesired spore formers were germinated and inhibit the germination of spores of *B.thuringiensis*. Later, samples were treated with 80 R"C for 3 min in Hot air oven to eliminate germination of vegetative cells of non sporulated bacterium and inoculum spreaded on T3 plates (Tryptose-2.0 g;Tryptone-3.0gYeast extract -1.5 g; Magnesium chloride -0.005g ; Agar - 15.0 g; Distilled water- 1000ml;pH-6.8). The colonies which had shown creamy white, circular and fried egg identified as *B.thuringiensis*.

Quantification of toxicity by bioassay is the way to assess the potency of isolates. Standard leaf dip method developed by Shelton *et al*. (1993) was adopted. Individual isolates of *B.thuringiensis* were streaked on T3 mediumplates and incubate at 37 R"C for 24 h. Later, loopful of culture was added to T3 broth and kept for sporulation under shaking condition for three days. Castor leaf disc of nine cm diameter was dipped into broth containing 0.1 per cent of Tween80 and leaf disc were air dried. The 20 third instar larvae were released for one replication and three replications were maintained with HD1(*B. thuringiensis* sub sp. *kurstaki*) as a reference strain. The leaf disc dipped in distilled water served as a control along with Tween80-0.1per centas one of the treatment. Larval mortalities were recorded after 24h at regular intervals up to seven days at a uniform dose of 1×10^{12} CFU ml^{-1}(Colony forming unit per ml). Cumulative mortalities were calculated and LT_{50} values were determined by probit analysis(Finney, 1984).

The toxicity of *B.thuringiensis*was a dose dependant phenomenon and dose acquired was directly proportional to the quantity of food consumed (Murali Krishna *et al*., 2018). Among 62 soil samples from different mandals, 15 isolates *viz*., KV_2S_1, KV_2S_3, CV_1S_1, CV_1S_2, CV_1S_3,CV_2S_3, EV_1S_1,$MPrV_2S_1$, MKV_2S_1, MKV_2S_3, MCV_1S_1, MCV_1S_2, MCV_1S_3, MCV_2S_3, MEV_1S_1 were identified as *B.thuringiensis* isolates.

A total of 15 isolates with median lethal time ranged from131.68to 194.42 hpi withfiducial limits of 120.06-146.87to 162.34- 199.09hpi. The reference strain HD1was recorded with LT_{50}value of 106.76hpi along with fiducial limits of 102.14-111.41hpi. The fastest lethal action to kill 50% of the population was recorded in five isolates *viz*., KV_2S_1 (131.68hpi) followed byMCV_1S_2(133.44hpi), MKV_2S_3(137.61hpi), MCV_2S_3 (143.29hpi) and MCV_1S_3 (145.46hpi) and slowestLT_{50} was observed with isolate, MCV_1S_1 (194.429hpi), followed byCV_1S_1(186.42hpi)and MEV_1S_1(176.45hpi) were more potent on third instar larvae of *S. litura*. The slowest lethal action was observed in isolates, MCV_1S_1(194.42hpi)(Table 1).

Similar results were also observed in the findings of Praveen *et al*. (2014), who isolated the five *B. thuringiensis* isolates from eleven different cropping ecosystem of Raichur, Karnataka. LT_{50} of isolates, BGC-1 (46.97 to 169.60 h), GBP-2 (51.22 to 226.50 h), KMP (55.76 to140.94 h), BGC-2 (57.23 to 177.28 h) and BGM-2 (59.85 to 130.62 h) were

Table 1. Lethal time mortality response of *S. litura* to the native isolates of *B.thuringiensis*

Isolate	Chi-square ($\chi 2$)	Regression equation	LT_{50} (hpi) at 1×10^{12} CFU ml^{-1}	Fiducial limits (hpi)	R^2
HD1	1.804	Y= -13.569+6.690X	106.76	102.14-111.41	0.998
KV_2S_1	12.792	Y= -13.893+6.555X	131.68	120.06-146.87	0.941
KV_2S_3	9.442	Y= -10.842+4.815X	178.48	155.29-238.13	0.902
CV_1S_1	9.661	Y= -10.385+9.574X	186.42	159.40-264.73	0.893
CV_1S_2	8.813	Y= -12.231+5.604X	152.16	138.40-175.81	0.971
CV_1S_3	7.888	Y= -12.089+5.485X	159.99	150.20-173.96	0.954
CV_2S_3	6.623	Y= -11.816+5.282X	172.64	160.64-192.15	0.944
EV_1S_1	9.196	Y= -11.865+5.354X	164.37	147.06-200.37	0.914
$MPrV_2S_1$	8.671	Y= -11.897+5.423X	156.18	141.33-183.12	0.977
MKV_2S_1	8.845	Y= -12.552+5.792X	146.97	134.44-166.88	0.984
MKV_2S_3	11.56	Y= -13.263+6.201X	137.61	125.52-155.02	0.949
MCV_1S_1	8.673	Y= -9.911+4.330X	194.42	164.55-282.88	0.982
MCV_1S_2	12.521	Y= -13.594+6.396X	133.44	121.59-149.47	0.947
MCV_1S_3	8.72	Y= -13.008+6.015X	145.46	133.61-163.63	0.965
MCV_2S_3	9.062	Y= -13.258+6.149X	143.29	131.75-160.49	0.958
MEV_1S_1	8.016	Y= -10.872+4.839X	176.45	162.34- 199.09	0.965

recorded on *Plutella xylostella*, whereas, the reference strain HD1 recorded with the LT_{50} value ranged from 41.98 to 130.62 h. The studies of the Murali Krishna *et al*. (2018) revealed the lethal times of four isolates, F493, F468, N30 and N115 were 78.52 h, 74.28 h, 95.70 h and 88.68 h respectively against *S. litura* were more pathogenic among 21 isolates.

The five isolates, KV_2S_1(131.68hpi) ,MCV_1S_2(133.44hpi), MKV_2S_3(137.61hpi), MCV_2S_3 (143.29hpi) and MCV_1S_3(145.46hpi) of *B. thuringiensis* were more pathogenic to third instar larvae of *S.litura* compared to the remaining identified isolates (KV_2S_3, CV_1S_1, CV_1S_2, CV_1S_3,CV_2S_3, EV_1S_1,$MPrV_2S_1$, MKV_2S_1, MCV_1S_1 and MEV_1S_1).

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