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In vitro evaluation of native *Bacillus thuringiensis* (Berliner) isolates against *Spodoptera litura* (Fabricius)

Hareesh Shiralli, Basavaraj Kalmath, Prabhuraj A, Arunkumar Hosmani and Ayyanagouda Patil

Abstract
Nineteen native *Bacillus thuringiensis* (*Bt*) strains isolated from soil samples were evaluated against II instar larvae of *Spodoptera litura* (Fab.) at College of Agriculture, Bheemarayanagudi, University of Agricultural Sciences, Raichur during 2018-19. The laboratory bioassays revealed that II instar *S. litura* larvae treated with 19 native *Bt* isolates showed mortality in range of 43.33 to 100% during preliminary screening. Highest mortality of 100% was recorded in HD-1 reference strain, which was on par with the native *Bt* isolates, BGC-1(96.66%), GBP-2(93.33%) and GHB-1(86.66%). Next best treatment was found to be *Bt* isolate BGC-2 which resulted in 83.33 per cent mortality. Lowest mortality (43.33 %) was recorded in *Bt* strain GHM-2 and no mortality was recorded in untreated control against II instar larvae of *S. litura* during preliminary screening. 89.47% of the *Bt* isolates (17/19) tested against II instar *S. litura* recorded more than 50% mortality. Median lethal concentration of the native isolates (BGC-1, GBP-2 and GHB-1) of *Bt* which recorded more than 85 per cent mortality in preliminary screening revealed that the toxicity of three potential *B. thuringiensis* isolates against second instar larvae of *S. litura* ranged from 2.89 to 21.31µg/ml. Among three potential isolates tested, isolate BGC-1 showed the least LC_{50} value of 5.24µg/ml and it was comparable to the reference strain HD1 with the LC_{50} value of 2.89µg/ml, this was followed by GBP-2 (8.65µg/ml) and GHB-1 (21.31µg/ml).

Keywords: *Bacillus thuringiensis*, LC_{50}, mortality and *Spodoptera litura*

1. Introduction
The tobacco caterpillar, *Spodoptera litura* (Lepidoptera: Noctuidae) is a polyphagous pest with high mobility and reproductive capacity widely distributed throughout tropical and temperate Asia, Australia and Pacific islands (Mohammad Monobrullah and Uma Shankar, 2008) [14]. *Spodoptera litura* (Fab.) devastates a large host range of more than 120 host plants (Ramana *et al*., 1988) [17]. Indiscriminate use of chemical insecticides to control this pest has resulted in resistance to chemical insecticides, resurgence and deleterious effects to environment and non-target organisms. In recent years microbial insecticides have become a viable alternative to control lepidopteran pests particularly *S. litura*. One of the most important insect pathogens in the world today is the bacteria *Bacillus thuringiensis* accounting for 1-2% of the global insecticide market (Lambert and Peferoen, 1992) [9]. Quantification of the toxicity by insect bioassays is the only way to assess the potency of a strain for pest control. Keeping the importance of *Bt* in view, the isolated native *Bt* strains from different zones of Karnataka were evaluated against *S. litura* under *in vitro* conditions (Saroja, 2017) [19].

2. Materials and Methods
2.1. Mass rearing of tobacco caterpillar, *Spodoptera litura*
Field collected larvae were brought to laboratory and maintained on healthy castor leaves till pupation. Newly formed pupae were collected daily and kept in separate petridishes. Pupae were separated into males and females based on their genital structure and maintained separately in petridishes. Castor plants were grown in pots caged with mosquito net, then the petriplates containing male and female pupae of *S. litura* were kept inside the cage. After adult emergence, food was provided with cotton dipped in 10 per cent honey solution. Every

alternate day, fresh honey solution was prepared and soaked in a cotton wad and provided in the petriplates. Eggs laid on castor leaves were collected in petridishes and kept in BOD incubator for emergence. The emerged neonate larvae along with castor leaves were transferred to a rearing box (18 x 11 cm) containing young castor leaves (natural host for maintaining healthy culture of *S. litura*) and reared till larvae reach second instar. Mass rearing was carried out in insect growth chamber having 26 ± 2 ^{0}C temperature and 65 ± 10 per cent relative humidity.

2.2. Maintenance of *Bacillus thuringiensis* culture

The studies were carried out at College of Agriculture, Bheemarayanagudi Karnataka, India during 2018-19. Already available nineteen *Bt* strains were used for bioassay along with reference strain *B. thuringiensis* sub sp *kurstaki* (HD1) to ascertain their insecticidal activity. Individual isolates were streaked on plain luria agar plates and incubated overnight at 37 ^{0}C. One loop of overnight cultures was inoculated in luria broth and kept for sporulation under shaking condition at 28 ^{0}C for 24h.

2.3. Preparation of *B. thuringiensis* suspension for preliminary bioassay

B. thuringiensis isolates were grown in 100 ml of Luria broth (Sambrook and Russell, 2001) [18] and incubated for five days at 30 ^{0}C (Ozkan *et al*., 2003) [15]. Cultures were centrifuged at 10,000 rpm for 10 minutes at 4 ^{0}C. The supernatant was discarded, the pellet was resuspended in 1ml sterile distilled water. The pellet was washed twice with sterile distilled water to remove the traces of supernatant. One gram of pellet was diluted and thoroughly mixed with 5ml sterile distilled water to conduct initial bioassay.

2.4. Preliminary bioassay against *S. litura*

The semisynthetic diet (Sambrook and Russel, 2001[18]) was poured as a thin layer into 12 celled multi cavity trays, with approximately 4 ml per well with a surface area of 3.14 cm^2. The bacterial suspension containing Tween-80 (0.02%) at 146 µl was overlaid on the diet surface in each well for all concentrations and kept for one hour. One pre-starved (4 h) second instar larva was released in each well. A total of 40 larvae were used for each concentration @ 10 larvae/ replication (4 replications including control). These trays were kept in an insectary at 25±1^{0}C, 70±5.0 per cent relative humidity (RH) and with light: dark as 16:8 hours. The observation on mortality was recorded at 1, 2, 3, 4 and 5 days after treatment. In addition, an untreated check was also maintained in order to get corrected mortality. The per cent mortality was calculated as per Abbott's, 1925[1] using the standard formula (Chandrasekaran *et al*., 2015) [3].

$$\text{Per cent mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

2.5 Bioassay of lyophilized promising native isolates of *B. thuringiensis* against *S. litura*

The *B. thuringiensis* isolates which recorded more than 85 per cent mortality in preliminary screening were further tested at different concentrations against second instar larvae of *S. litura* in comparison with reference HD1 strain of *B. thuringiensis*. Lyophilized *B. thuringiensis* technical powder was serially diluted for conducting bioassay against second instar larvae of *S. litura* at six different concentrations. The larvae fed with sterile distilled water served as control. Four replications were maintained for each concentration. The procedure mentioned in preliminary bioassay was also followed for this experiment. The observations on larval mortality were recorded at an interval of 24 hr for five days. Concentrations and mortality data were used for determination of median lethal concentration (LC_{50}).

3. Statistical analysis

The data generated from the laboratory experiments were subjected to statistical analysis by Completely Randomized Design (CRD) as described by Yates, 1937 [22]. After arcsine transformation, data was subjected to analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT) (Duncan, 1955) [5]. Median lethal concentration (LC_{50}) were calculated by probit analysis (Finney, 1971) [6].

4. Results and Discussion

When the II instar larvae of *S. litura* treated with native *Bt* strains, the larvae showed normal activity up to 24 h of inoculation. On the second day the rate of feeding diminished. The larvae became sluggish, turned black with signs of vomiting and diarrhoea. Finally larvae died within 2 to 4 days due to starvation and septicemia. The insecticidal activity of *Bt* is mainly due to its ability to synthesize parasporal crystal. When susceptible insect ingests α endotoxins of 130 kDa, they are solubilized and proteolytically digested to yield the active toxin form of molecular weight 60 to 70 kDa polypeptide. The toxin bind to protein receptors in the epithelial insect midgut and produces pores leading to the loss of normal membrane function. As a result epithelial cells lyse and feeding activity is paralyzed, finally insect die due to starvation and septicemia (Luthy and Wolfersberger, 2000) [10].

Table 1: Preliminary evaluation of native isolates of *B. thuringiensis* against II instar larvae of *S. litura* in laboratory

Sl. No.	Isolates	Per cent mortality				
		1 DAT	2 DAT	3 DAT	4 DAT	5 DAT
1	HD1(ref.)	13.33 (20.95) ^{a}	30.00 (31.62) ^{a}	56.66 (44.46) ^{a}	80.00 (55.41) ^{a}	100 (71.80) ^{a}
2	BGC-1	10.00 (18.13) ^{b}	26.66 (29.76) ^{b}	56.66 (44.46) ^{a}	76.66 (53.60) ^{ab}	96.66 (68.08) ^{b}
3	BGC-2	13.33 (20.95) ^{a}	23.33 (27.68) ^{c}	43.33 (38.32) ^{b}	63.33 (47.45) ^{bc}	83.33 (56.99) ^{abc}
4	RCM-1	3.33 (10.04) ^{d}	13.33 (20.95) ^{f}	26.66 (29.76) ^{cde}	36.66 (35.04) ^{fg}	50.00 (41.39) ^{e}
5	RCM-2	13.33 (20.95) ^{a}	23.33 (27.68) ^{c}	40.00 (36.61) ^{bc}	60.00 (45.95) ^{c}	76.66 (53.60) ^{bcd}
6	KMF	0.00 (0.00) ^{e}	6.66 (12.08) ^{h}	16.66 (23.44) ^{e}	33.33 (35.27) ^{fg}	60.00 (45.95) ^{def}
7	GHM-1	0.00 (0.00) ^{e}	6.66 (12.08) ^{h}	16.66 (23.44) ^{e}	33.33 (35.27) ^{fg}	50.00 (41.39) ^{e}
8	GHM-2	3.33 (10.04) ^{d}	13.33 (20.95) ^{f}	26.66 (29.76) ^{cde}	36.66 (35.04) ^{fg}	43.33 (38.32) ^{e}
9	GBP-1	13.33 (20.95) ^{a}	26.66 (29.76) ^{b}	43.33 (38.32) ^{b}	63.33 (47.45) ^{bc}	76.66 (53.60) ^{bcd}
10	GBP-2	13.33 (20.95) ^{a}	23.33 (27.68) ^{c}	40.00 (36.61) ^{bc}	63.33 (47.45) ^{bc}	93.33 (66.26) ^{ab}
11	KMS-1	0.00 (0.00) ^{e}	13.33 (20.95) ^{f}	23.33 (27.68) ^{de}	40.00 (36.61) ^{efg}	53.33 (42.89) ^{ef}
12	KMS-2	10.00 (18.13) ^{b}	20.00 (25.75) ^{d}	33.33 (35.27) ^{bcd}	53.33 (42.89) ^{cde}	73.33 (52.10) ^{cd}

13	MDS-1	0.00 (0.00) [e]	6.66 (12.08)[h]	16.66 (23.44) [e]	26.66 (29.76) [g]	53.33 (42.89) [ef]
14	MDS-2	3.33 (10.04)[d]	13.33 (20.95) [f]	23.33 (27.68) [de]	36.66 (35.04) [fg]	60.00 (45.95) [def]
15	GHB-1	13.33 (20.95) [a]	23.33 (27.68) [c]	33.33 (35.27) [bcd]	56.66 (44.46) [cd]	86.66 (58.81) [abc]
16	GHB-2	3.33 (10.04)[d]	10.00 (18.13) [g]	20.00 (25.75) [de]	30.00 (31.62) [fg]	50.00 (41.39) [e]
17	MDC	3.33 (10.04)[d]	13.33 (20.95) [f]	23.33 (27.68) [de]	33.33 (35.27) [fg]	46.66 (39.89) [e]
18	MDH	6.66 (12.08)[c]	16.66 (23.44) [e]	26.66 (29.76) [cde]	43.33 (38.32) [def]	73.33 (52.10) [cd]
19	GPP-1	0.00 (0.00) [e]	6.66 (12.08)[h]	16.66 (23.44) [e]	30.00 (31.62) [fg]	70.00 (50.52) [cde]
20	GHP	3.33 (10.04)[d]	13.33 (20.95) [f]	26.66 (29.76) [cde]	43.33 (38.32) [def]	76.66 (53.60) [bcd]
21	Control	0.00 (0.00) [e]	0.00 (0.00) [i]	0.00 (0.00) [f]	0.00 (0.00) [h]	0.00 (0.00) [g]
S. Em ±		0.30	0.47	0.78	0.96	1.09
CD @1%		0.66	1.82	1.99	2.17	2.63
CV (%)		1.96	2.08	2.74	3.69	4.22

4.1. Preliminary screening against *S. litura*

The screening of nineteen native isolates of *B. thuringiensis* as well as reference strain HD-1 against second instar larvae of *S. litura* was recorded from one to five days after feeding. In general, the mortality of *S. litura* was increased with incubation period and maximum mortality was recorded after 5 days of feeding. Among the twenty isolates and reference strain HD-1, four (HD-1, BGC-1, GBP-2 and GHB-1) isolates caused 86 to 100 per cent mortality, six isolates (BGC-2, RCM-2, GBP-1, KMS-2, MDH and GHP) caused 71 to 85 per cent mortality, five isolates (KMF, KMS-1, MDS-1, MDS-2 and GPP-1) caused 51 to 70 per cent mortality, four isolates (RCM-1, GHM-1, GHB-2 and MDC) caused 46 to 50 per ce nt mortality and the remaining one isolate (GHM-2) caused less than 45 per cent mortality against second instar larvae of *S. litura* (Table 1and Fig. 1).

The cumulative mortality of second instar larvae of *S. litura* ranged from 0 to 13.33 per cent after one day of exposure. Significantly highest mortality of 13.33 per cent was recorded in isolates BGC-2, RMC-2, GPB-1, GPB-2, and GHB-1 as well as in reference strain HD-1. The isolates BGC-1 and KMS-2 were recorded the mortality of 10.00 per cent each and they were on par with each other. The isolate MDH was on par with 6.66 per cent mortality. Zero mortality was recorded in isolates KMF, GHM-1, KMS-1, MDS-1, GPP-1 and the control at the end of one day exposure (Table 1and Fig. 1).

At two days after exposure, cumulative mortality ranged from 6.66 to 30.00 per cent. Significantly highest mortality of 30.00 per cent was recorded in reference strain HD-1. Among the native isolates, highest mortality of 26.66 per cent was recorded in the isolates BGC-1 and GBP-1 followed by BGC-2, RMC-2, GBP-2 and GHB-1 which recorded mortality of 23.33 per cent each. Isolate KMS-2 was recorded 20.00 per cent mortality followed by 16.66 per cent in isolate MDH. Isolates RCM-1, GHM-2, KMS-1, MDS-2, MDC and GHP were recorded 13.33 per cent mortality each followed by the isolate GHB-2 was recorded 10.00 per cent mortality. The lowest mortality of 6.66 per cent was recorded in isolates KMF, GHM-1, MDS-1 and GPP-1 and there was zero per cent mortality in the control treatment (Table 1and Fig. 1).

After 3 days of exposure, cumulative mortality in all treatments ranged from 16.66 to 56.66 per cent. Significantly highest mortality of 56.66 per cent in isolate BGC-1 as well as in reference strain HD-1 was observed. Among the native isolates, significantly highest mortality of 43.33 per cent was recorded in BGC-2 and GBP-1 followed by 40.00 per cent in isolates RCM-2 and GBP-2. The next mortality of 33.33 per cent was registered in isolates GHB-1 and KMS-2 each. The other isolates *viz*., RCM-1, GHM-2, MDH and GHP recorded 26.66 per cent mortality each fallowed by 23.33 per cent mortality in KMS-1, MDS-2 and MDC each. The isolate GHB-2 showed mortality of 20.00 per cent. The remaining isolates KMF, GHM-1, MDS-1 and GPP-1 were recorded 16.66 per cent mortality, all these were on par with each other and zero per cent mortality was observed in the control(Table 1and Fig. 1).

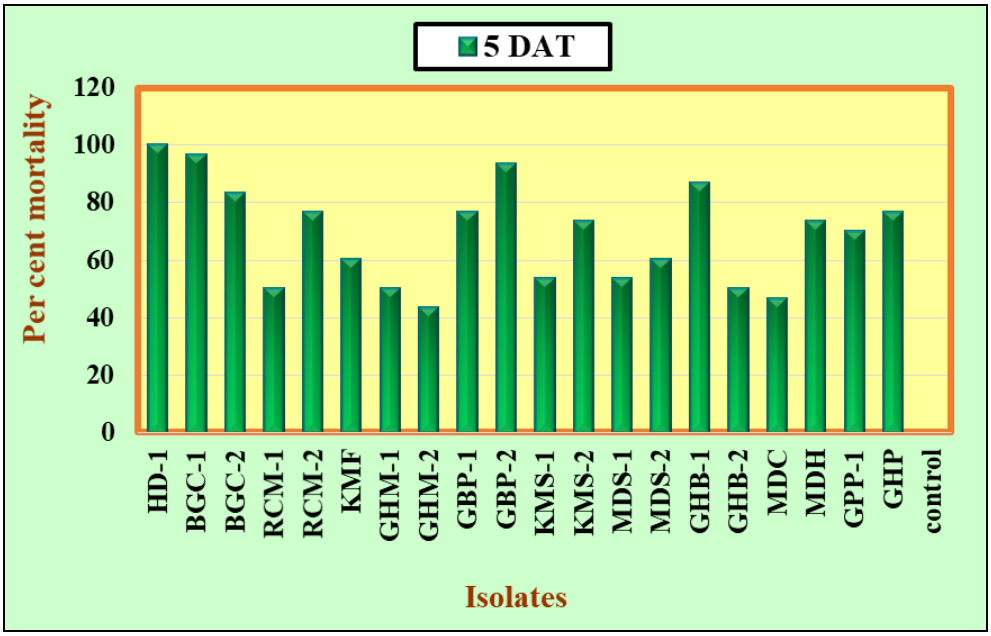

Fig 1: Preliminary evaluation of native isolates of *B. thuringiensis* against second instar larvae of *S. litura* in laboratory

The cumulative mortality ranged from 26.66 to 80.00 per cent after 4 days of exposure. The reference HD-1 strain was recorded significantly highest mortality of 80.00 per cent. Among the native isolates, BGC-1 with 76.66 per cent mortality was recorded followed by 63.33 per cent in the isolates BGC-2, GBP-1 and GBP-2 all these were on par with each other. The isolates RCM-2, GHB-1 and KMS-2 which showed mortality of 60.00 per cent, 56.66 per cent and 53.33 per cent respectively, all these were on par with each other. The remaining isolates like MDH and GHP which recorded 43.33 per cent mortality, KMS-1 which showed 40.00 per cent followed by RCM-1, GHM-2 and MDS-2 which recorded mortality of 36.66 per cent. The per cent mortality of 33.33 was recorded in KMF, GHM-1 and MDC followed by 30.00 per cent mortality was registered in GHB-2 and GPP-1 The lowest mortality of 26.66 per cent mortality was recorded in MDS-1 and zero mortality was observed in the control treatment (Table 1and Fig. 1).

At 5 days after treatment, pathogenicity increased in all isolates where in mortality rate increased to 100.00 per cent in reference strain HD-1. Among the native isolates, significantly highest mortality of 96.66 per cent was recorded in isolate BGC-1 followed by the 93.33 per cent and 86.66 per cent in the isolates GBP-2 and GHB-1 respectively, all these were on par with each other. The next highest mortality of 83.33 per cent was recorded in isolate BGC-2 followed by 76.66 per cent mortality was observed in isolates RCM-2, GBP-1 and GHP. The isolates like KMS-2 and MDH was recorded 73.33 per cent and GPP-1 was showed 70.00 per cent mortality. The other isolates such as KMF and MDS-2 were recorded the mortality of 60.00 per cent followed by mortality of 53.33 per cent was observed in KMS-1 and MDS-1 each. The other isolates, like RCM-1, GHM-1 and GHB-2 were registered mortality of 50.00 per cent. The lowest mortality of 46.66 per cent and 43.33 per cent was recorded in isolates such as MDC and GHM-2, respectively; they were statistically on par with each other. There was no mortality in the control treatment (Table 1and Fig. 1).

Table 2: Concentration mortality response (LC_{50}) of *S. litura* to lyophilized native isolates of *B. thuringiensis*

Sl. No.	Isolates	LC_{50} (µg/ml)	Fiducial limit		Regression equation	χ^2 value
			Lower limit	Upper limit		
1	HD-1	2.89	1.01	8.28	Y = 4.790206 + 0.4590316x	7.95
2	BGC-1	5.24	1.82	15.03	Y = 4.680234 + 0.4443457x	4.82
3	GBP-2	8.65	2.83	26.40	Y = 4.602741 + 0.4239566x	2.06
4	GHB-1	21.31	7.20	63.10	Y = 4.389631 + 0.4593534x	3.69

4.2. Median lethal concentration (LC_{50}) of potential lyophilized *B. thuringiensis* isolates against *S. litura*

In general, the medium lethal concentrations (LC_{50}) of promising isolates were ranged from 5.24 to 21.31 µg/ml. The LC_{50} value of reference strain HD-1 was found to be lowest 2.89 µg/ml with fiducial limit ranging from 1.01 to 8.28 values, which was comparable with the BGC-1 isolate was 5.24 µg/ml with fiducial limit ranging from 1.82 to 15.03 value. This was followed by GBP-2 with 8.65 µg/ml with fiducial limit ranging from 2.83 to 26.40 value and GHB-1 with 21.31 µg/ml with fiducial limit ranging from 7.20 to 63.10 values (Table 2, Fig.2).

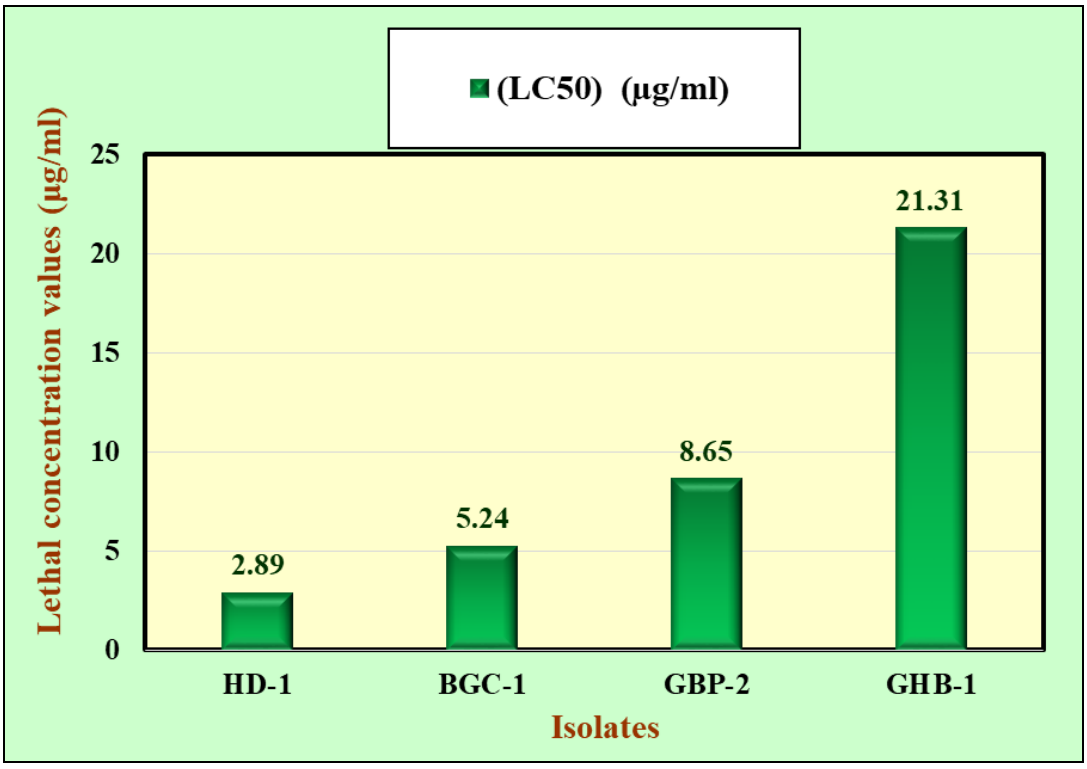

Fig 2: Lethal concentration (LC_{50}) values of potential native isolates of *B. thuringiensis* against second instar larvae of *S. litura*

In support of our results, preliminary assays performed with spore crystal mixture by Lalitha *et al*., 2012 [8] also reported that the native *B. thuringiensis* strains cause mortality ranged from 16.67 per cent to 94.44 per cent after 98 hr of feeding.

Similarly, Saroja, 2017) [19] revealed that the twenty *B. thuringiensis* strains were toxic to second instar larvae of *H. armigera* and causes the mortality ranging from 36.66 per cent to 100.00 per cent after 120 hr of feeding. Patel *et al*., 2009[16] reported that the seven *B. thuringiensis* strains were toxic to second instar larvae of *H. armigera* and it causes the mortality ranging from 20.00 to 80.00 per cent after 48 hr of infestation. Xavier *et al.*, 2007[21] reported that 30 *B. thuringiensis* strains showed 40.00 to 100.00 per cent mortality against second instar larvae of *H. armigera*.

The results of our present findings are in conformity with the results of Chatterjee, 2008 [4]. They have evaluated the efficacy of *B. t.* var. *kurstaki* (*B.t.k*) at concentrations of 5500 and 3200 IU/mg against 2nd instar larvae of *S. litura* and *H. armigera* under laboratory conditions. *B. t.* var. *kurstaki* at 5500 IU/mg recorded 86.49 and 68.59 per cent mortality of *S. litura* and *H. armigera*, respectively.

According to Merdan *et al.*, 2010 [12], the LC_{50} for the two isolates Shae.5 and Dkah.1 with respect to *S. littoralis* were 103.55 and 127.74 µg/ml respectively. Values for LC_{90} determined were found to be 797.57 µg/ml and 1055.75 µg/ml for the two isolates, respectively.

Lakshminarayana and Sujatha, 2005 [7] collected 21 strains of *B. thuringiensis* from USA. These proteins were tested at 125, 250 and 500 µg/ml against 5 days old *S. litura*. larval mortality at 6 DAT at higher concentration (500 mg/ml) was 0 to 50 per cent.

The variation in LC_{50} values for different *B. thuringiensis* strains was also reported by several workers. Silva *et al.*, 2004 [20] reported most efficient strain against *S. frugiperda* in their study was the strain S701 that showed a value of LC_{50} around 2.6 times smaller than HD-1 strain. Moar *et al*., 1989 [13] found that LC_{50} values for NRD-12 and HD-1 against *S. exigua* were 20.80 µg/ml and 49.30 µg/ml of diet, respectively. Cry 1C and Cry 1E were highly toxic to first instar larvae of *S. exempta* with LC_{50} values of 0.38 and 0.33 µg/ml, respectively. The LC_{50} of Cry 1E was 10-fold higher for third instar larvae of *S. litura* (Bai *et al*., 1993) [2]. Mc Carthy, 1994[11] reported that lines of *Spodoptera* sp. were more susceptible to *B. t.* var. *kurstaki* than *B. t.* var. *aizawai*. The LC_{50} values were 0.2 to 0.8 µg/ml and 2.5 to 3.7 µg/ml, respectively.

5. Conclusion

Mortality increased with incubation period and maximum mortality was recorded at 5 days after feeding. Three isolates were found to be potential *viz*., (BGC-1, GBP-2 and GHB-1) caused mortality ranged from 85 to 100 per cent against second instar larvae of *S. litura*. Median lethal concentration (LC_{50}) of three potential isolates against second instar larvae of *S. litura* ranged from 5.24 to 21.31 µg/ml. Isolate BGC-1 showed the least LC_{50} value of 5.24 µg/ml.

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