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Bioefficacy of wettable powder formulation of native *Bacillus thuringiensis* isolate against major Lepidopteran pests in the Laboratory

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ABSTRACT

Biofilm based antagonists were evaluated for growth promotion and soil borne disease The bioefficacy of the *Bacillus thuringiensis* (Berliner) Cry proteins in filed condition can be improved by developing formulation. In the presence study, we have developed and evaluated native *Bacillus thuringiensis* (Bt) isolate for the pathogenic activity against major lepidopteran pests *viz., Helicoverpa armigera, Spodoptera litura* and *Plutella xylostella.* The bioassay of the *Bacillus thuringiensis* crystal spore mixture against *H. armigera* registered 36.67 to 96.67 per cent mortality at 120 h after feeding. Reference strain HD1 showed highest mortality of 100 per cent. Lyophilized native *B. thuringiensis* (BGC-1) and *B. thuringiensis* (HD-1) were tested against *H. armigera*, recorded mortality ranging from 16.67 to 96.67 per cent. The LC_{50} value of *B. thuringiensis* (BGC-1) and *B. thuringiensis* (HD-1) were 6.08 and 9.18 ng/ml respectively. Bioefficacy of WP formulations of BGC-1 and HD-1 were recorded ranging from 32.50 to 95.00 and 35.00 to 97.50 percent mortality respectively at different concentration. The same WP formulation was also tested against *S. litura* and *P. xylostella,* recorded 87.50 and 95.00 per cent mortality for *S. litura* and *P. xylostella,* respectively. The study concludes that native isolate BGC-1 was found to be promising against major lepidopteran pests.

Keywords: *Bacillus thuringiensis,* Lyophilization, *Helicoverpa armigera, Spodoptera litura, Plutella xylostella,* Bioassay, Wettable powder formulations.

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INTRODUCTION

Yield losses due to insect damage are a major problem in agricultural and horticultural crops. There are different modes of avoiding these losses. Micro-organism like viruses, bacteria, fungi, protozoa and mites are employed to control insect attack on crops. Prokaryotic bacteria are unicellular organisms with varied size from less than 1µm to several µm in which are either spherical, rod and spiral in shape. Most of the entmopathogenic bacteria occur under the families Bacillaceae, Pseudomonadaceae, Enterobacteriaceae and Sterptococcaceae (Tanada and kaya, 1993). Members of Bacillaceae, particularly *Bacillus* spp. have received maximum attention as microbial control agents. The classical example of such bacterial pathogen *Bacillus thuringiensis* Berliner (Eubacteriales: Bacillaceae) occupy 90 per cent of world biopesticides market and is pathogenic to more than 525 insects species belonging to various orders but mainly to Lepidoptera, Diptera, Coleoptera and Hymenoptera (Sunderbabu, 1985). *Bacillus thuringiensis* is a soil borne, rod shaped, facultative, gram-positive, aerobic, endospore forming bacterial species, which is highly pathogenic to insects. *B. thuringiensis* was first time discovered in Japan in 1901 from infected larvae of silk worm, *Bombyx mori* by Ishiwata, and later it was isolated and identified by Berliner in 1911 (Baum *et al.,* 1999). *B. thuringiensis* first become available as a commercial insecticide in 1938 and then 1950s in France and United States, respectively. Commercial formulations based

on *B. thuringiensis* were introduced in 1960s. This was envisaged as an alternative to conventional insecticides. To date more than 30 products of *B. thuringiensis* are available.

The bioefficacy of the *B. thuringiensis* in the laboratory is prominently high compare to field condition, mainly because of inactivation of cry toxins when expose to temperature, sunlight. The bioefficacy of the *B. thuringiensis* could be improved by developing the formulation. Wettable powder formulation is one of the best formulations to improve the efficacy of the *B. thuringiensis* in the field condition. With this background hereby we made an attempt to to evaluate native *B. thuringiensis* isolate, develop and evaluate the wettable powder formulation against major lepidopteran pests.

MATERIALS AND METHODS.

Site of experiment

All experiments in this research work were carried out in the Department of Agricultural Entomology, AC, B'gudi and NFSM lab, UAS Raichur during the year of 2016-17.

Maintenance of *Bacillus thuringiensis* culture

The native isolated *B. thuringiensis* strains along with the reference HD-1 strains were taken from Department of Agricultural Entomology, Bheemarayana gudi. *B. thuringiensis* strains were sub cultured on Luria agar medium at 30°C for 48 h and stored at 4ºC for the further studies.

Mass rearing of *Helicoverpa armigera, Spodoptera litura* and *Plutella xylostella*

Mass rearing of test insects viz., cotton bollworm, *Helicoverpa armigera* and tobacco caterpillar, *Spodoptera litura* were reared in the laboratory on the chickpea based artificial diet till pupation (Kranthi, 2005). Newly formed pupae were collected on daily basis and they were sexed into male and female pupae based on their genital structure and maintained. After the emergence, adults were introduced into ovipositional chamber. An each alternative day, fresh honey solution was prepared and soaked in a cotton wad and hanged in ovipositional chamber. Later the ovipositional chamber was covered with sterile black muslin cloth and secured with rubber band. Similarly, fresh black muslin cloth was provided an each alternate day for oviposition. Later, egg mass along with muslin cloth was transferred to a rearing box with moist sponge pad to facilitate emergence of neonate larvae. After emergence, the neonate larvae released on breadbox containing artificial diet for two days and then transferred to multi cavity tray containing artificial diet. Second instar larvae were used for further laboratory bioassay studies (Vimaladevi and vineela, 2014).

Diamond back moth was mass cultured in the laboratory following the method described by Liu and Sun (1984) with little modification. The larvae collected from the field were reared separately on cabbage leaves raised in green house under insecticide-free conditions. Pupae thus obtained were kept in a petriplate and placed in a cage of 25 cm^3 for adult emergence. When moths started emerging, mustard seedlings were provided for oviposition. Mustard seedlings were raised in plastic cups of 6 cm height and 4.5 cm diameter filled with coco peat in cups under natural conditions. Within 4-5 days after germination, they were placed in the oviposition cage and replenished at 24 h interval. Ten per cent honey solution containing multivitamin powder was provided for the adults as artificial food through cotton swab kept in a sterilized petriplate. The moth laid eggs on both sides of cotyledons. The cups with eggs were transferred to plastic tubs (45x30x15 cm) for mass rearing. Eggs hatched in 2-3 days and neonates mined the mustard cotyledons and fed on them. When the cotyledons were completely consumed, larvae were transferred to fully expanded cabbage leaves with petiole covered in wet cotton swab to maintain leaf turgidity. Third instar larvae were used for further laboratory bioassay studies (Vastrad, 2000).

Preliminary bioassay of native *B. thuringiensis* isolates against *H. armigera*

B. thuringiensis isolates were grown in 100 ml of Luria broth (Sambrook and Russell, 2001) and incubated for five days at 30 ºC (Ozkan *et al.,* 2003). Cultures were centrifuged at 10,000 rpm for 10 minutes at 4 ºC. 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. The diet 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 larvae were released in each well. A total of 40 larva was used for each concentration at 10 larvae/ replication (4 replication including control). These trays were kept in an insectary at 25±1°C, 70±5.0 per cent relative humidity (RH) and with light: dark as 16:8 hours. The observation on mortality were recorded at 24, 48, 72, 96 and 120 hrs after treatment (Vimaladevi and vineela, 2014). The per cent mortality was calculated as per Abbott's (1925) using the standard formula (Chandrasekaran *et al.,* 2015).

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

Lyophilization and bioassay of promising native *B. thuringiensis* isolate against *H. armigera*

The *B. thuringiensis* isolates which recorded more than 85 per cent mortality was further taken for lyophilization. Lyophilization was done at the department of Biotechnology, GKVK UAS Bangalore. Lyophilized *B. thuringiensis* technical powder was serially diluted for conducting bioassay at six different concentrations. The methodology for bioassay studies is same as mentioned above. The observations on larval mortality were recorded at an interval of 24 h for five days. Concentrations and mortality data were used for determination of median lethal concentration (LC_{50}).

Development and evaluation of Wettable powder formulation against *H. armigera*

The Wettable Powder (WP) formulation was prepared in a aseptic condition. A concentrated 2 gm WP formulation was prepared by mixing 0.4 gm lyophilized powder with the other ingredients (Gouder, 2011). Initially 0.4 gm lyophilized powder and 0.26 gm boric acid both are mixed thoroughly with the help of mortar and pestle. Add 10 mg of sucrose, 60µl of tween-80 and 40µl of triton X-100 and finally 15 mg of silica gel were added mixed thoroughly with the help of mortar and pestle and the prepared formulation was stored at 4 ºC used for bioassay.

The WP formulation of *B. thuringiensis* (BGC-1 and HD-1) were tested against *H. armigera* with different dosages *viz.,* 0.5 gm/l, 1 gm/l, 1.5 gm/l, 2gm/l and 2.5 gm/l of distilled water. The methodology for bioassay studies is same as mentioned above. The observations on larval mortality were recorded at an interval of 24 h for five days. Concentrations and mortality data were used for determination of median lethal concentration (LC_{50}). The insecticidal potency (ITU) of the sample was calculated by using the standard formula (Dulmage *et al.*, 1971).

$$\text{ITU of sample} = \frac{\text{LC50 of standard} \times \text{Reference standard ITU}}{LC_{50}\text{ of sample}} \times 100$$

Bioassay of WP formulations against *S. litura* and *P. xylostella*

The effective dosage of prepared WP formulations were evaluated against *S. litura* and *P. xylostella.* The second instar larvae of *S. litura* and third instar larvae of *P. xylostella* were taken for the treatment.

For *S. litura,* the methodology for bioassay studies is same as that of *H. armigera*. WP formulation containing Tween-80 (0.02%) at 146 µl was overlaid on the diet surface in each well and kept for one hour. One pre-starved (4 hours) second instar larvae were released in each well. A total of 40 larvae were used for each concentration at 10 larvae/ replication. These trays were kept in an insectary at 25±1°C, 70±5.0 per cent relative humidity (RH) and with light: dark as 16:8 hours. The observation on mortality were recorded at 24,

48, 72, 96 and 120 hrs after treatment (Vimaladevi and vineela, 2014).

For DBM, the mustard were grown in pots, leaves were used for conducting bioassay. The leaves were washed with 0.1 per cent formaldehyde, transfer the leaves serially to water blanks to remove the traces of formaldehyde, air dried, prepared the WP formulation and leaves were dipped in each 20 ml suspension for a period of 3 min, air dried and 4 replication were maintained including control. The petiole was moistened with wet cotton, wrapped with aluminum foil over the cotton. Ten larvae were released to each container, with four replications maintained. The number of dead larvae was recorded at 24, 48 and 72 h after treatment.

Statistical analysis

Analysis of the bioassay results was carried out for the dose mortality response (LC_{50}) using the method proposed by Finney (1952) with the help of MLP package. The data generated from the laboratory experiments were subjected to statistical analysis by Completely Randomized Design (CRD) described by Yates (1937).

RESULTS

Preliminary screening of native *B. thuringiensis* isolates against *H. armigera*

Preliminary assays performed with spore-crystal mixture of native *B. thuringiensis* isolates against second instar larvae of *H. armigera*. The mortality of *H. armigera* was ranged from 0 to 10.00 per cent after 24 h of exposure. Significantly highest mortality of 10.00 per cent was recorded in isolates BGC-1, GBP-2 as well as reference strain HD1. Maximum mortality was observed in reference strain HD-1 (33.33%) followed by native isolate BGC-1 (26.67 %) and 23.33 per cent in both GBP-2 and BGM-2 at 48 h after exposure. The cumulative mortality was ranged from 20.00 to 63.33 per cent and 26.67 to 90.00 per cent in all the treatments after 72 h and 96 h of exposure, respectively. At 120 h, pathogenicity increased in all isolates wherein mortality rate increased to 100.00 per cent in reference strain HD-1. Among the native isolates, significantly highest mortality of 96.67 per cent was recorded in isolate BGC-1 followed by the 90.00 per cent and 86.67 per cent in the isolate GBP-2 and BGM-2 respectively, both are on par with each other and the lowest mortality 46.67 per cent and 36.67 per cent in isolate GPP-1 and KMS-1, respectively. More than 85 per cent mortality after 120 h of exposure was recorded by three isolates *viz.,* BGC-1, GBP-2 and BGM-2 (Table. 1). The crystal spore mixture (CSM) of these isolates were further used for lyophilization and lyophilized powder was used in the bioassay studies to confirm its effectiveness against *H. armigera.*

Standardization of dosages of native *B. thuringiensis* isolates

Bioassay of potential *B. thuringiensis* isolates

The results of the bioassay on concentration mortality response of second instar larvae of *H. armigera* to with the selected promising isolates (BGC-1, GBP-2 and BGM-2) and the reference strain HD1 revealed that mortality increases with increased in the concentration.

HD-1

The mortality of second instar larvae of *H. armigera* was ranged from 0 to 6.67 per cent after 24 h of exposure. Significantly highest mortality of 6.67 per cent was recorded in the concentrations of 1000, 100 and 10 ng/ml. The cumulative mortality was ranged from 3.33 to 23.33 per cent, 6.67 to 43.33 per cent and 13.33 to 76.67 per cent, respectively. At 120 h of exposure, highest mortality of 96.67 per cent was recorded in 1000 ng/ml concentration followed by 76.67 per cent mortality in 100 ng/ml (Table 2).

BGC-1

The mortality of second instar larvae of *H. armigera* was ranged from 0 to 6.67 per cent after 24 h of exposure. The highest mortality of 6.67 per cent was recorded in the concentration of 1000 ng/ml followed by 3.33 per cent in 100, 10 and 1 ng/ml concentrations. At 48 h, 72 h and 96 h of after treatment, the cumulative mortality was ranged from 3.33 to 16.67 per cent, 6.67 to 36.67 per cent and 10.00 to 63.33 per cent, respectively. The cumulative mortality was ranged between 16.67 to 93.33 per cent at 120 h of exposure.

Table 1. Per cent mortality of native *B. thuringiensis* isolates against *Helicoverpa armigera* at different time interval

Isolates	Per cent mortality at				
	24 h	**48 h**	**72 h**	**96 h**	**120 h**
HD-1 (ref)	10.00 a	33.33 a	63.33 a	90.00 a	100.00 a
MDS-1	6.67 b	16.67 e	36.67 d	50.00 def	66.67 ef
MDS-2	0.00 d	6.67 h	20.00g	33.33ij	56.67 fgh
GBP-1	3.33 c	10.00 g	20.00 g	30.00jk	50.00 gh
GBP-2	10.00 a	23.33 c	43.33 c	63.33 c	90.00 c
GPP-1	0.00 d	6.67 h	20.00 g	30.00jk	46.67 hi
KMS-1	0.00 d	6.67 h	16.67 g	26.67 k	36.67 i
KMS-2	3.33 c	13.33 f	33.33 de	46.67efg	63.33 efg
KMF	0.00 d	10.00 g	26.67f	46.67efg	56.67 fgh
BGC-1	10.00 a	26.67 b	53.33 b	73.33 b	96.67 b
BGC-2	3.33 c	16.67 e	36.67d	56.67 d	83.33 cd
GHB-1	0.00 d	10.00 g	30.00ef	40.00 hi	60.00 efgh
GHB-2	3.33 c	13.33 f	36.67 d	50.00 def	70.00 ef
GHP	3.33 c	16.67 e	33.33 de	43.33 fgh	60.00 fgh
RCM-1	0.00 d	13.33 f	30.00 ef	36.67 hi	56.67 efgh
RCM-2	6.67 b	16.67 e	33.33de	53.33 de	73.33 de
GHM-1	0.00 d	13.33 f	30.00 ef	43.33 fgh	66.67 ef
GHM-2	3.33c	13.33 f	26.67 f	36.67 hi	56.67fgh
MDC	6.67 b	20.00d	33.33de	43.33 fgh	63.33 efg
BGM-2	6.67 b	23.33c	43.33 c	66.67 c	86.67 c
Control	0.00 d	0.00 i	0.00 h	0.00 l	0.00 j
S. Em±	**0.20**	**0.49**	**0.78**	**0.96**	**1.08**
CD @ 1%	**0.77**	**1.87**	**2.99**	**3.67**	**4.14**

The values represented by same alphabet are statistically on par with each other by DMRT.

concentration. The lowest mortality of 23.33 and 16.67 per cent was recorded in 0.1 and 0.01 ng/ml concentration, respectively (Table 2). The highest mortality of 93.33 per cent was recorded in 1000 ng/ml concentration followed by 73.33 per cent mortality in 100 ng/ml

GBP-2

Initially at 24 h, all the concentrations recorded larval mortalities ranging from 0 to 6.67 per cent. Significantly highest mortality were recorded in the concentration of 1000 and 100 ng/ml. The cumulative mortality was ranged from zero to 16.67 per cent, 6.67 to 33.33 per cent and 10.00 to 60.00 per cent at 48 h, 72 h and 96 h of exposure, respectively. At 120 h, the cumulative mortality was ranged from 13.33 to 86.67 per cent. Significantly highest mortality of 86.67 per cent was recorded in 1000 ng/ml concentration followed by 66.67 per cent mortality in 100 ng/ml concentration (Table 2).

BGM-2

At 24 h exposure, the concentrations like 1000, 100 and 10 ng/ml were recorded highest mortality of 3.33 per cent. Significantly highest mortality of 13.33%t, 40% and 66.67% was recorded in 1000 ng/ml concentration at 48 h, 72 h and 96 h, respectively. At 120 h, the cumulative mortality was ranged from 10.00 to 86.67 per cent. Concentration 1000 ng/ml quoted significantly highest mortality of 86.67 per cent followed by 60 per cent mortality in 100 ng/ml concentration. The remaining concentrations were recorded mortality ranged from 10.00 to 33.33 per cent (Table 2).

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Table 2. Per cent mortality of potential lyophilized *Bacillus thuringiensis* powder against *H. armigera* at different time interval

Isolates	Concentration (ng/ml)	Per cent mortality at				
		24 h	**48 h**	**72 h**	**96 h**	**120 h**
HD - 1 (ref)	1000	6.67 (14.96) a	20.00 (26.57) b	43.33 (41.17) a	76.67 (61.11) a	96.67 (79.48) a
	100	6.67 (14.96) a	23.33 (28.88) a	40.00 (39.23) a	63.33 (53.73) b	76.66 (61.11) b
	10	6.67 (14.96) a	13.33 (21.42) c	23.33 (28.88) b	33.33 (35.27) c	40.00 (39.23) c
	1.00	3.33 (10.52) b	10.00 (18.43) d	16.67 (24.09) c	26.67 (31.09) c	33.33 (35.27) d
	0.10	3.33 (10.52) b	6.67 (14.96) e	10.00 (18.43) d	16.67 (24.09) d	26.67 (31.09) d
	0.01	0.00 (0.00) c	3.33 (10.52) f	6.67 (14.96) e	13.33 (21.42) d	20.00 (26.57) d
	Control	0.00 (0.00) c	0.00 (0.00) i	0.00 (0.00) f	0.00 (0.00) e	0.00 (0.00) e
S. Em ±		**0.11**	**0.25**	**0.48**	**1.01**	**1.27**
CD @ 1 %		**0.49**	**1.05**	**2.05**	**4.28**	**5.34**
BGC - 1	1000	6.67 (14.96) a	16.67 (24.09) a	36.67 (37.27) a	63.33 (54.74) a	93.33 (75.03) a
	100	3.33 (10.52) b	13.33 (21.42) b	26.67 (31.09) b	53.33 (46.91) b	73.33 (58.91) b
	10	3.33 (10.52) b	10.00 (18.43) c	16.67 (24.09) c	26.67 (31.09) c	40.00 (39.23) c
	1.00	3.33 (10.52) b	6.67 (14.96) d	13.33 (21.42) d	23.33 (28.88) d	33.33 (35.27) c
	0.10	0.00 (0.00) c	3.33 (10.52) e	10.00 (18.43) e	20.00 (26.57) e	23.33 (28.88) d
	0.01	0.00 (0.00) c	3.33 (10.52) e	6.67 (14.96) f	10.00 (18.43) f	16.67 (24.09) d
	Control	0.00 (0.00) c	0.00 (0.00) f	0.00 (0.00) g	0.00 (0.00) g	0.00 (0.00) e
S. Em ±		**0.09**	**0.25**	**0.47**	**1.15**	**1.50**
CD @ 1 %		**0.41**	**1.06**	**1.98**	**4.84**	**6.34**
GBP – 2	1000	6.67 (14.96) a	16.67 (24.09) a	33.33 (35.27) a	60.00 (50.76) a	86.67 (68.58) a
	100	6.67 (14.96) a	13.33 (21.42) b	26.67 (31.09) b	46.67 (43.09) b	66.67 (54.74) b
	10	3.33 (10.52) b	10.00 (18.43) c	20.00 (26.57) c	33.33 (35.27) c	36.67 (37.27) c
	1.00	0.00 (0.00) c	3.33 (10.52) d	10.00 (18.43) d	20.00 (26.57) d	30.00 (33.21) cd
	0.10	0.00 (0.00) c	3.33 (10.52) d	10.00 (18.43) d	16.67 (24.09) d	23.33 (28.88) d
	0.01	0.00 (0.00) c	0.00 (0.00) e	6.67 (14.96) e	10.00 (18.43) e	13.33 (21.42) e
	Control	0.00 (0.00) c	0.00 (0.00) e	0.00 (0.00) f	0.00 (0.00) f	0.00 (0.00) f
S. Em ±		**0.07**	**0.17**	**0.45**	**0.80**	**1.12**
CD @ 1 %		**0.31**	**0.73**	**1.90**	**3.36**	**4.73**
BGM – 2	1000	3.33 (10.52) a	13.33 (21.42) a	40.00 (39.23) a	66.67 (54.74) a	86.67 (68.58) a
	100	3.33 (10.52) a	10.00 (18.43) b	26.67 (31.09) b	46.67 (43.09) b	60.00 (50.76) b
	10	3.33 (10.52) a	10.00 (18.43) b	16.67 (24.09) c	26.67 (31.09) c	33.33 (35.27) c
	1.00	0.00 (0.00) b	3.33 (10.52) c	6.67 (14.96) d	13.33 (21.42) d	23.33 (28.88) d
	0.10	0.00 (0.00) b	3.33 (10.52) c	6.67 (14.96) d	13.33 (21.42) d	16.67 (24.09) e
	0.01	0.00 (0.00) b	0.00 (0.00) d	3.33 (10.52) e	6.67 (14.96) e	10.00 (18.43) f
	Control	0.00 (0.00) b	0.00 (0.00) d	0.00 (0.00) f	0.00 (0.00) f	0.00 (0.00) g
S. Em ±		**0.05**	**0.18**	**0.48**	**0.81**	**1.06**
CD @ 1 %		**0.21**	**0.76**	**2.03**	**3.44**	**4.47**

Note : Figures in the parentheses are "arcsine" transferred values. The values represented by same alphabet are statistically on par with each other by DMRT

LC_{50} of potential *B. thuringiensis* isolates

In general, the median lethal concentrations (LC_{50}) of promising isolates were ranged from 9.14 to 36.23 ng/ml. The LC_{50} value of reference strain HD-1 was found to be lowest (6.08 ng/ml with fiducial limit ranging from 2.28 to 16.20 value), which was comparable with the BGC-1 was isolate (9.14 ng/ml with fiducial limit ranging from 3.50 to 23.85 value). This was followed by GBP-2 (19.21 ng/ml with fiducial limit ranging from 6.80 to 54.31 value) and BGM-2 (36.23 ng/ml with fiducial limit ranging from 213.90 to 94.45 value) (Fig. 1).

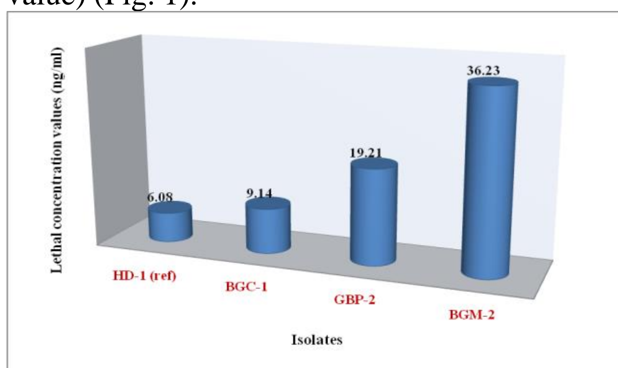

Fig. 1: Lethal concentration values (LC_{50}) of potential *Bacillus thuringiensis* isolates against second instar larvae of *Helicoverpa armigera*

Evaluation of *B. thuringiensis* WP formulations against *H. armigera.*

The wettable powder formulation was prepared by using promising native strain BGC-1 and reference strain HD-1. The feeding cessation was observed within hour after 120 h of treatment, per cent mortality recorded was ranged from 35 to 97.50 per cent against second instar larvae of *H. armigera* (Table 3). At 120 h of exposure, the cumulative mortality was ranged between 35.00 to 97.50 per cent. Significantly highest mortality of 95.00 per cent was recorded in the 2.5 g/l concentration in the reference strain HD-1. Where as in the native isolate BGC-1, significantly highest mortality of 95.00 per cent was recorded in the 2.5 g/l. The bioassay studies revealed that larval mortality increases with increased in the concentration and the exposure time.

Evaluation of *B. thuringensis* WP formulation against *S. litura* and *P. xylostella.*

The promising strains of BGC-1 and HD1 WP formulations and commercial formulations Dipel and NBAIR *Bt* were evaluated against second instar larvae of *S. litura* and third instar larvae of *P. xylostella.* The mortality of second instar larvae of *S. litura* was ranged from 0 to 15.00 per cent after 24 h of exposure (Table 4). As time of exposure increase the mortality also increases hence, at 120 h of exposure, the cumulative mortality ranged between 82.50 to 90 per cent. The mortality of 90.00 per cent, 87.50 per cent, 85.00 per cent and 82.50 per cent were recorded in the Dipel, BGC-1 WP formulation, HD-1WP and NBAIR *Bt* all these were statistically on par with each other and there was no significant difference between all these formulations. Finally, the mortality recorded after 120 h of treatment was ranged from 82.50 to 90.00 per cent and zero per cent mortality was observed in the control treatment.

The mortality of third instar larvae of *P. xylostella* ranged from 22.50 to 37.50 per cent after 24 h of exposure (Table 5). At 48 h of exposure, significantly highest mortality of 70.00 per cent was recorded in the NBAIR *Bt*. The cumulative mortality ranged from 95.00 to 100.00 per cent after 72 h of exposure. The mortality 100.00 per cent was recorded in Dipel followed by 97.50 per cent in HD-1 WP formulation and 95 per cent in BGC-1 WP formulation and NBAIR *Bt* alone. These all formulations are statistically on par with each anther and they do not differ significantly among the treatments.

DISCUSSION

The use of biological control products are increased in modern agriculture after chemical pesticide. But, the market of biopesticides still limiting because, they are very expensive and non reliable, it is similar with the *B. thuringiensis.* A report by Praveen, 2015 identified twenty native *B. thuringiensis* to be toxic to *P. xylostella* and it caused mortality ranged from 13.33 to 90.00 per cent 72 h after feeding. Preliminary assays performed with spore-crystal mixture of native *B. thuringiensis* isolates against second instar larvae of *H. armigera*. In general the larval

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Table 3. Per cent mortality of WP formulation of *B. thuringiensis* against *H. armigera* at different time interval

Isolates	Concentration g/l	Per cent mortality at				
		24 h	48 h	72 h	96 h	120 h
HD - 1	0.5	0.00 (0.00) d	10.00 (18.43) e	22.5 (28.31) c	30.00 (33.21) e	35.00 (36.27) e
	1.0	2.50 (9.09) c	17.50 (24.72) d	27.5 (31.62) c	42.50 (40.68) d	50.00 (45.00) d
	1.5	5.00 (12.92) b	22.50 (28.31) c	45.00 (42.13) b	55.00 (47.86) c	65.00 (53.72) c
	2.0	7.5 (15.89) a	30.00 (33.21) b	50.00 (45.00) b	72.500 (58.37) b	85.00 (67.21) b
	2.5	7.5 (15.89) a	37.50 (37.76) a	62.50 (52.23) a	82.50 (65.27) a	97.50 (80.90) a
	Control	0.00 (0.00) d	0.00 (0.00) f	0.00 (0.00) d	0.00 (0.00) f	0.00 (0.00) f
S.Em ±		**0.11**	**0.43**	**0.84**	**1.22**	**1.68**
CD @ 1%		**0.45**	**1.80**	**3.49**	**5.09**	**7.02**
BGC - 1	0.5	0.00 (0.00) c	5.00 (12.92) e	17.5 (24.72) e	27.50 (31.62) d	32.50 (34.75) d
	1.0	2.50 (9.09) b	10.00 (18.43) d	22.5 (28.31) d	37.50 (37.76) c	47.50 (43.56) c
	1.5	2.50 (9.09) b	12.50 (20.70) c	27.5 (31.62) c	47.50 (43.56) b	55.00 (47.86) c
	2.0	5.00 (12.92) a	17.50 (24.72) b	37.5 (37.76) b	55.00 (47.86) b	72.50 (58.37) b
	2.5	5.00 (12.92) a	22.50 (28.31) a	45 (42.13) a	67.50 (55.24) a	95.00 (77.07) a
	Control	0.00 (0.00) c	0.00 (0.00) f	0.00 (0.00) f	0.00 (0.00) e	0.00 (0.00) e
S.Em ±		**0.08**	**0.34**	**0.78**	**1.17**	**1.56**
CD @ 1%		**0.33**	**1.43**	**2.36**	**4.89**	**6.52**

Note : Figures in the parentheses are "arcsine" transferred values. The values represented by same alphabet are statistically on par with each other by DMRT

Table 4 . Per cent mortality of *B. thuringiensis* formulation against *S. litura* at different time intervals

Different strains of *Bt*	Per cent mortality at				
	24 h	48 h	72 h	96 h	120 h
BGC – 1	7.50 (15.89) c	20.00 (26.56) c	37.50 (37.76) c	65.00 (53.72) b	87.50 (69.29) a
HD – 1	12.50 (20.70) b	40.00 (39..23) a	50.00 (45.00) ab	70.00 (56.78) ab	85.00 (67.21) a
Dipel	15.00 (22.78) a	30.00 (33.21) b	47.50 (43.56) b	72.50 (58.37) ab	90.00 (71.56) a
NBAIR *Bt*	15.00 (22.78) a	32.50 (34.75) b	57.50 (49.31) a	75.00 (60.00) a	82.50 (65.27) a
Control	0.00 (0.00) d	0.00 (0.00) d	0.00 (0.00) d	0.00 (0.00) c	0.00 (0.00) b
S. Em ±	**0.32**	**0.65**	**1.12**	**1.44**	**1.83**
CD @ 1%	**1.32**	**2.69**	**4.66**	**6.01**	**7.61**

Note : Figures in the parentheses are "arcsine" transferred values. The values represented by same alphabet are statistically on par with each other by DMRT

mortality in the experiment ranges from zero to 10.00 per cent upto 24 h but increased with increase in time. The maximum mortality was registered between 36.67 and 100.00 per cent at 120 h after feeding. More than 85 per cent mortality after 120 h of exposure was recorded by three isolates *viz.,* BGC-1, GBP-2 and BGM-2. It was observed that the mortality was very low up to 24 h of feeding. This might be due to the fact that *B. thuringiensis* being stomach poison, it has to enter to in the midgut of insect, where it gets dissolved in the alkaline pH, releasing delta endotoxin (Heimpel and Angus, 1959) which may take more than 24 h time to kill the insect.

In support of our results, Preliminary assays performed with spore crystal mixture by Lalitha *et al.* (2012) reported that the native *Bt* strains cause mortality ranged from 16.67 per cent to 94.44 per cent after 98 h against second instar larvae of *H. armigera*. Similarly, Patel *et al.* (2009) revealed that the seven *Bt* strains

Table 5. Per cent mortality of *B. thuringiensis* formulation against *P. xylostella* at different time intervals.

Different strains of *Bt*	Per cent mortality at		
	24 h	**48 h**	**72 h**
BGC – 1	22.50 (28.31)d	57.50 (49.31) b	95.00 (77.07) b
HD – 1	37.50 (37.76)a	60.00 (50.76) b	97.50 (80.90) b
Dipel	32.50 (34.75)b	67.50 (55.76)ab	100.00 (90.00) a
NBAIR *Bt*	25.00 (30.00)c	70.00 (56.78) a	95.00 (77.07) b
Control	0.00 (0.00) e	0.00 (0.00) c	0.00 (0.00) c
S. Em ±	**0.67**	**1.29**	**2.14**
CD @ 1%	**2.79**	**5.38**	**8.92**

Note : Figures in the parentheses are "arcsine" transferred values. The values represented by same alphabet are statistically on par with each other by DMRT

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 h of infestation.

The concentration mortality response data on *B. thuringiensis* isolates showed a progressive increase in the dose required to cause 50.00 per cent mortality reported by earlier workers in bioassay studies with Entomopathogens (Sureen *et al.,* 1983; Pojas and Calilung, 1984 and Zaz, 1989). Among three native isolates (BGC-1, GBP-2 and BGM-2) of *B. thuringiensis*, isolate BGC-1 was more virulent with 96.67 per cent mortality and the lowest LC_{50} value of 9.14 ng/ml. The presents finding are in conformity with the results of Malik *et al.,* 2013 reported the LC_{50} value of 9 ng/mg of artificial diet was exhibited by local *Bt* isolates HW 4.4 and INS 2.25 against second instar larvae of *H. armigera.*

The differences in the efficacy of different isolates of *B. thuringiensis* has been suggested to be due to the difference in the carbohydrate affinity of the domain II which results in variable binding specificity with the receptors at the brush border membrane of the insect larvae, causing difference in toxicity of the cry protein (Burton *et al.,* 1999). According to Ashfaq *et al*. (2001), found that the length of the larval developmental period increased linearly with an increase in feeding time.

B. thuringiensis has been extensively used for four decades in biopesticidal formulations due to its safe environmental and human health records and solid formulation was more effective than liquid formulation (Lalitha *et al*., 2012), they are being sold as either wettable powder or granuals or suspension of spores (Bernhard and Utz, 1995). Hence, Wettable powder was selected for evalution of efficacy and standardize the native *Bacillus thuringiensis* against *H. armigera.*

In the present study, efficient isolate BGC-1 and the reference strain HD1 were used for the preparation of wettable powder (WP) formulations and the formulations were also tested for their efficacy against *H. armigera*. The cumulative mortality was ranging from 35.00 to 95.70 per cent after 120 h of feeding at concentration of 2.5 g/l with the LC_{50} value of 0.9 g/l and assigned a biopotency of 18,000 ITU/g against second instar larvae of *H. armigera.* In BGC-1 WP formulation, the cumulative mortality was ranging from 32.50 to 95.00 per cent after 120 h of feeding at concentration of 2.5 g/l with the LC_{50} value of 1.5 g/l and assigned a biopotency of 15428.57 ITU/g against second instar larvae of *H. armigera*. Similarly, the LC_{50} value of HD-263 was 0.53µg/g and the assigned 42,264 IU/mg of biopotency was reported by Navon

et al., 1990. Biopotency of 53000 IU/mg in Delfin, 17600 IU/mg in Dipel and 15000 IU/mg in Centari were found aganist *P. xylostella* (Justin *et al.,* 2001). The LC_{50} of Bactosporine was 0.97-1.35 g/l and Dipel was 1.441.65 g/l reported by Sharma and Reddy (1993). Ajanta *et al.* (1999) found that larval mortality was ranged from 12.69 to 76.77 per cent in Biobit and 20.68 to 74.56 per cent in both Biolep and Dipel. Teera-arunsiri *et al.* (2003) have reported development of *Bt* ssp. *aizawai* based WP formulation with 55 per cent suspendibility, 24s wetting time and 5.69×10^4 CFU/ml of LC_{50} value against *Spodoptera exigua* larvae.

Dipel performed best and it recorded the highest mortality of 90.00 per cent after 120 h followed by BGC-1WP (87.50%) against *S. litura* neonate larvae at 120 h. The similar results were also observed by the earlier workers Pandey *et al*. (2009) reported highest mortality of 73.33 per cent against third instar *S. litura* larvae was recorded in Biolep at 10 per cent concentration. Similary in *P. xylostella,* Dipel performed best and it recorded the highest mortality of 100 per cent followed by HD-1 WP formulation caused 97.50 per cent at 72 h against third instar larvae of *P. xylostella* (Table 6). Similar results were obtained by Tabashnik *et al.,* 1993 and Shelton *et al.,* 1993. Singh *et al.* (2003) recorded per cent morality 66.67 per cent against fourth larval instars of *P. xylostella* of Biolep formulation and conclueded *Bt* formulation are effective. The present experiment thus, reported development of *B. thuringiensis* formulations was efficient to control the *S. litura* and *P. xylostella.*

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