Journal of Biological Control, 32(3): 179-186, 2018, DOI: 10.18311/jbc/2018/21661

Research Article

Evaluation of UV protectants for wettable powder formulation of native *Bacillus thuringiensis* (Berliner) isolate against *Helicoverpa armigera* (Hubner) in the Laboratory

SAROJA[1], BASAVARAJ KALMATH[1*], M. BHEEMANNA[2] and A. PRABHURAJ[2]

[1]*College of Agriculture, Bheemarayanagudi, Shahapur, Yadgiri - 585287, Karnataka, India*

[2]*College of Agriculture, UAS Raichur - 584104, Karnataka, India*

**Corresponding author E-mail:* bskalmath@gmail.com

ABSTRACT: Radiation severely inactivates the potency of *Bacillus thuringiensis* spores and crystals present in sprayed formulations leading to decreased efficacy in field. Incorporation of UV protectants to biopesticides is one of the methods to protect against radiation damage. Keeping this as objective, a native isolate BGC-1 was selected for preparation and evaluation of wettable powder formulation against second instar larvae of *Helicoverpa armigera*. Median lethal concentration of the BGC-1 was 1.05 g/l and assigned biopotency value was 15428.57 ITU/g. UV protectants *viz*., melanin, para-amino benzoic acid, polyvinyl alcohol and Congo red were evaluated by exposing formulated solution to sunlight at different intervals of time. Among four UV protectants, melanin showed an excellent UV protecting ability with the mortality of 86.67 per cent and, 116.49 µg/ml of crude protein at 5 h sunlight exposure with temperature of 43.6°C and light intensity of 4.93×10^5 lux followed by 80.00 per cent mortality, 1.74×10^8 CFU/ml and 109.40 µg/ml of crude protein in para-amino benzoic acid UV protectant. Next best UV protectant was congo red with the mortality of 73.33 per cent and, 90.76 µg/ml of crude protein and 1.26×10^8 CFU/ml. It is concluded that melanin was found to be an effective UV protectant for *B.* thuringiensis WP formulations against *H. armigera*.

KEY WORDS: *Bacillus thuringiensis, Helicoverpa armigera,* lyophilized powder, WP formulation, UV protectants

(Article chronicle: Received: 04-06-2018; Revised: 09-08-2018; Accepted: 10-09-2018)

INTRODUCTION

Bacillus thuringiensis Berliner (Bt) is being used as a biological control agent (Magda and Bendary, 2006). Most entomopathogens are not stable against natural environmental stresses such as Ultra Violet (UV) radiation, rain and temperature. Radiation is the main limitation that severely inactivates the potency of *B. thuringiensis* crystals against different insect pests. Formulation of biopesticides by adding various UV protectants, adjutants or phago stimulants, is one of the methods to overcome these limitations (Amoura *et al.,* 2009), however, in adjutant-based formulations, the contact between the protectants and active ingredient is not always adequate (Brar *et al.,* 2006).

Helicoverpa armigera commonly known as gram pod borer, American bollworm of cotton, tomato fruit borer, maize cob borer and sunflower head borer is a polyphagous pest. This pest causes extensive losses in cotton, pulses, oilseeds and certain vegetable crops in India. In India, total losses in both pulses and cotton exceed $530 million annually and the extent of losses in chickpea and pigeonpea worldwide has been estimated over $927 million annually (Ragesh *et al*., 2015). Due to excessive and injudicious use of chemical pesticides this pest has started developing resistance to most chemical pesticides. Hence, *B. thuringiensis* is an effective insecticide, relatively harmless to natural enemies, safe to the higher animals and environmentally acceptable.

Limited study on the effect of *B. thuringiensis* formulations on the *H. armigera* is a serious drawback in the field of *H. armigera* management. There is an urgent need for screening of newer adjuvants that may be incorporated in formulations to improve the efficacy of *B. thuringiensis.* Hence, the present study was taken to develop effective *B. thuringiensis* formulation against *H. armigera.* The main objective was to compare the protective effect of the UV protectants on the stability of formulations exposed to UV radiation. Spore viability and the crude protein content of WP formulations also evaluated.

MATERIALS AND METHODS

Site of experiment

All experiments in this research work were carried out in the Department of Agricultural Entomology, A.C, Bheemarayanagudi and NFSM lab, UAS Raichur during the year of 2016–17.

Maintenance of *Bacillus thuringiensis* culture

The native *Bacillus thuringiensis* strain BGC-1 along with the reference HD1 strain were taken from Department of Agricultural Entomology, AC, B' gudi. *B. thuringiensis* strains were sub cultured on Luria agar medium at 30ºC for 48 h and stored at 4ºC for the further studies.

Lyophilization of bacterial pellets

The bacterial cells were suspended in five liter Luria broth for five days at 30°C. The turbid solution was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and pellets were subjected to lyophilization to technical powder as shown in flowchart. The lyophilized powder was stored at 4ºC. Lyophilized *B. thuringiensis* technical powder was used to prepare six different concentrations of (1×10^{-4}, 1×10^{-5}, 1×10^{-6}, 1×10^{-7}, 1×10^{-8} and 1×10^{-9}) for conducting bioassay against second instar larvae of *Helicoverpa armigera*.

Mass rearing of *Helicoverpa armigera*

Mass rearing of cotton bollworm, *H. armigera* was 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 on 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 were 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.

Flow chart of lyophilization of *Bacillus thuringiensis*

B. thuringiensis pellet were transferred to the petriplate with sterilized the spatula and spread it uniformly

↓

Cover with aluminum foil and holes were made on foil

↓

Kept in deep freezer at - 20°C for 2-3 hr

↓

Again Kept in deep freezer at - 80°C for 2-3 hr

↓

liquid nitrogen of 10-20 ml was added

↓

After 10 min, petriplate were kept in a lyophilizer at - 40°C over night

↓

Next day morning, petriplate containing *B. thuringiensis* pellet flakes were grinded with help of mortar and pestle

Bioassay of promising native lyophilized *Bacillus thuringiensis* technical powder

The diet was poured as a thin layer into 12 celled multi cavity trays, 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 hours) second instar larvae were released in each well. A total of 40 larvae were used for each concentration @ 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 observations on mortality were recorded at 24, 48, 72, 96 and 120 hrs after treatment (Vimaladevi and Vineela, 2014). In addition, an untreated check was also maintained in order to get corrected mortality. The per cent mortality was calculated as per Abbott (1925) using the standard formula. Concentrations and mortality data were used for determination of median lethal concentration (LC_{50}).

Development of wettable powder formulation without UV protectant

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. 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 4ºC used for bioassay.

Evaluation of wettable powder formulation against *Helicoverpa armigera*

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, 2 gm/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).

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

Screening of different UV protectants for *Bacillus thuringiensis* formulations

Three different WP formulations were prepared with containing different UV protectants *viz.*, Melaninand Congo red along with control (without UV protectant). Effective dose of the

formulation was selected for further testing at different intervals of sunlight. This is to find out the efficacy of UV protectants to protect the toxin when exposed to sunlight. Fifty ml effective dosage of WP formulation with different UV protectants was prepared in 100 ml plastic cups, exposed to sunlight at different intervals of time (0h, 1h, 2h, 3h, 4h and 5h). Solar light intensity was measured by solar pyranometer on planer surface and it is designed to measure the solar radiation in W/m^2 then, it was converted to lux.

The exposed solution was fed to second instar larvae of *H. armigera* as mentioned above. The observations on larval mortality were recorded at an interval of 24 h for five days. Colony forming units and soluble protein of the solution were calculated.

Quantification of colony forming units (CFU/ml)

The total number of viable cells in the bacterial suspension was determined. The contents of the suspension was mixed thoroughly, one milligram of the sample was aseptically extracted and transferred into 1 ml of distilled water blank and 0.1 ml of the sample was as extracted and transferred into 0.9 ml of water blank. Serial dilutions were made and 100µL of suspension was plated on LA medium plates. These plates were incubated at 30ºC for 24 h in a BOD incubator. Colonies appearing on the plates were counted and CFU per milliliter in the bacterial suspension was calculated.

Quantification of protein by Lowry's method

The aromatic amino acids present in a protein like tyrosine, tryptophan react with phosphomolybdo - phosphor tungstate (FCR) reagent to produce a blue coloured complex at 660 nm. Soluble proteins were extracted from dissolving 0.2 gm of formulation using distilled water. One ml sample was taken in test tube and five ml of alkaline copper reagent was added and kept for 10 minutes. Then 0.5 ml of 1 N FCR was added and kept in dark place for 30 minutes. Per cent absorbance was read at 660 nm. Soluble proteins were calculated using Bovine Serum Albumin (BSA) standards (20–100 µg) (Lowry *et al*., 1951).

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 AND DISCUSSION

Standardization of dosages of native *Bacillus thuringiensis* isolates

Based on LC_{50} values established against *Helicoverpa armigera* by employing standard bioassay method the *Bacillus thuringiensis* isolates which caused higher mortality were selected for further studies. The concentration mortality response data showed a progressive increase in the dose required to cause 50 per cent mortality as reported by earlier workers in bioassay studies with entomopathogens (Zaz, 1989).

Among the three isolates (BGC-1, GBP-2 and BGM-2) native *B. thuringiensis* isolate BGC-1 was more virulent possessing lowest LC_{50} value of 9.14 ng/ml with fuducial limit ranging from 3.50 to 23.85 value and was comparable to the reference strain HD1 with LC_{50} value of 6.08 ng/ml with fiducial limit ranging from 2.28 to 16.20 value followed by the isolates GBP-2 (19.21 ng/ml with fiducial limit ranging from 6.80 to 54.31) and the isolate BGC-2 which showed highest LC_{50} value of 36.23 ng/ml with fiducial limit ranging from 13.90 to 94.45 (Table 1). As the technical powder concentration increased the mortality also increased in all the isolates. The presents finding are in conformity with the results of Malik *et al.* (2013) who have 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* and Lakshminarayana and Sujatha (2003) reported that Bt toxin was superior even at lower concentration (125 ng/cm^2) at 48h.

Contrastingly Sharma *et al.* (2014) reported that Bt technical powder caused high mortality of *H. armigera* with LC_{50} value of 12µg/ml. Similarly, the LC_{50} of pure crystals, pure spores and the spore-crystal complex were 22.1µg/ml, 23.2µg/ml and 20.2µg/ml, respectively against second instar larvae of *Spodoptera exigua* (Yang *et al.,* 2007). Praca *et al.,* 2013 reported that the LC_{50} of three *B. thuringiensis* strains S1905, S2122 and S2124 that were toxic to *Plutella xylostella* varied between 2.336 to 4.842 µg/ml. Degree of pathogenicity was varied with concentration of bacteria as well as the period of exposure (Savitri and Muralimohan, 2003). 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). Present findings showed that higher mortality was registered with increasing concentration of lyophilized powder and there was a direct relationship between mortality and concentration of lyophilized powder and increase in feeding period. According to Ashfaq *et al.* (2001) the length of the larval developmental period increased linearly with an increase in feeding time.

Evaluation of wettable powder formulation against *Helicoverpa armigera*

Bacillus thuringiensis has been extensively used for four decades in biopesticide 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 granules or suspension of spores (Bernhard and Utz, 1995) and commercially available primarily as WP formulations with effective dosage ranging

Table 1. Concentration mortality response (LC_{50}) of *Helicoverpa armigera* to lyophilized *Bacillus thuringiensis* powder

Sl. No.	Isolates	LC_{50} (ng/ml)	Fiducial limit		Regression equation	χ^2 value
			Lower	Upper		
1	HD-1 (ref)	6.08	2.28	16.20	Y = 4.60611 + 0.50347x	9.87
2	BGC-1	9.14	3.50	23.85	Y = 4.50634 + 0.51363x	5.18
3	GBP-2	19.21	6.80	54.31	Y = 4.38282 + 0.48077x	3.26
4	BGM-2	36.23	13.90	94.45	Y = 4.12841 + 0.55900x	2.56

Table 2. Concentration mortality response (LC_{50}) of *Helicoverpa armigera* to WP formulation of *Bacillus thuringiensis*

Sl. No.	Isolates	LC_{50} (g/l)	Fiducial limit		Regression equation	χ^2 value	Biopotancy (ITU/mg)
			Lower	Upper			
1	HD-1 (ref)	0.90	0.75	1.10	Y = 5.11411 + 2.77073x	8.38	18,000.00
2	BGC-1	1.05	0.87	1.28	Y = 4.94197 + 2.41427x	9.33	15428.57

from 1 to 2 kg/ha for management of lepidopteron pests (Vimala Devi and Vineela, 2014). The potency of bioassay for selecting *B. thuringiensis* preparation against agricultural insect pests was developed by Navon *et al.* (1990).

In the present study, isolate BGC-1 and the reference strain HD1 were used for the preparation of Wettable Powder (WP) formulations and the same were tested for their efficacy against *H. armigera*. In both WP formulations, the larval mortality in the experiment was low or nil upto 24 h but increased with time. The HD1 WP formulation elicited an LC_{50} value of 0.9 g/l and assigned a biopotency of 18,000 ITU/g against second instar larvae of *H. armigera.* The BGC-1 WP formulation exhibited an LC_{50} value of 1.5 g/l and assigned a biopotency of 15428.57 ITU/g (Table 2).

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 against *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). Similarly, a report by Kashyap and Amla, 2007 found that LC_{50} value for *H. armigera* was 0.04 µg of HD73, 0.031 µg of HD1, 0.011 µg of Dipel and 0.008 µg of HD1 Dipel.

Screening of different UV protectants for *Bacillus thuringiensis* WP formulations

Bacillus thuringiensis insecticides are adversely affected by the environmental factors and one major factor limiting the survival of spores and protein in fields appears to be components of sunlight, mainly UV-A (320–400 nm) and UV-B (280–320 nm) radiation. These wavelengths of radiation are responsible for the photo degradation and inactivation of various biopesticides under field conditions (Hadpad *et al.,* 2009).

In the present study, UV protectants like melanin and congo red were screened for *B. thuringiensis* WP formulations by exposing the solution to sunlight from 0 to 5 h. In the formulation without UV protectants, the per cent mortality recorded was 60.00 and number of colonies counted are 1.10×10^8 in (BGC-1) and 1.10×10^8 in (HD1) and crude protein content was 69.55 µg/ml (BGC-1) and 74.76 µg/ml (HD1) (Table 3). The mortality of 86.67 per cent in both formulation, 1.89×10^8 (BGC-1) and 2.01×10^8 (HD1) number of colonies and 116.49 µg/ml (BGC-1) and 113.97 µg/ml (HD1) of crude protein was recorded in melanin UV protectant (Table 4).

In congo red, 73.33 per cent mortality was recorded in both formulation with 1.26×10^8 (BGC-1) and 1.28×10^8 (HD1) number of colonies and 90.76 µg/ml (BGC-1) and 94.88 µg/ml (HD1) of crude protein (Table 5).

The exposure of *B. thuringiensis* solution to sunlight for 5 h resulted in 60 per cent reduction in larval mortality, number of colonies and protein content of native isolate BGC-1 and reference strain HD1 in the WP formulation without UV protectant but in melanin there was only 10 per cent and 20 per cent reduction and in Congo red 30 per cent reduction. Among formulations, melanin performed best.

The present findings are in comparison with Hadpad *et al.* (2009) and Zhang *et al.* (2016). The exposure of *B. sphaericus* ISPC-8 and 1593 spores to UV-B radiation for 6 h resulted in complete loss of spore viability and 50 per cent reduction in larvicidal activity (Hadpad *et al.,* 2008) and the formulations containing para-amino benzoic acid and congo red will protect the spore viability and larvicidal activity up to 168 h from UV B radiation (Hadpad *et al.,* 2009). Similarly, at 0.015 per cent melanin acts as UV protectant (Zhang *et al.,* 2016).

Table 3. Effect of sunlight and temperature on larvicidal activity, Colony count and Crude protein of native *Bacillus thuringiensis* strain BGC-1 and reference strain HD1 formulation against *Helicoverpa armigera* (without UV protectant)

Time exposure (h)	Native strain *B. thuringiensis* BGC-1 formulation					Reference strain HD1 formulation				
	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)
0	28.5	2.02×10^5	96.67 (79.48)a	2.42×10^8	164.91	29.0	2.04×10^5	96.67 (79.48)a	2.89×10^8	145.06
1	36.1	2.13×10^5	86.67 (68.58)b	2.03×10^8	122.10	34.2	2.06×10^5	86.67 (68.58)b	2.64×10^8	126.49
2	38.2	3.02×10^5	73.33 (58.91)c	1.80×10^8	111.15	37.4	3.00×10^5	83.33 (65.91)bc	2.37×10^8	118.78
3	39.1	3.70×10^5	73.33 (58.91)c	1.43×10^8	106.89	38.9	3.50×10^5	73.33 (58.91)cd	2.26×10^8	107.84
4	41.4	4.69×10^5	60.00 (50.76)d	1.23×10^8	81.77	42.0	4.52×10^5	60.00 (50.76)d	$1.19.\times10^8$	89.55
5	43.6	4.93×10^5	60.00 (50.76)d	1.08×10^8	69.55	43.5	4.86×10^5	60.00 (50.76)d	1.10×10^8	74.76
S. Em ±			1.75	0.04	2.23	S. Em ±		1.81	0.05	2.25
CD @ 1%			7.57	0.20	9.42	CD @ 1%		7.82	0.23	9.71

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

Table 4. Effect of sunlight and temperature on larvicidal activity, Colony count and Crude protein of native *Bacillus thuringiensis* strain BGC-1 and reference strain HD1 formulation against *Helicoverpa armigera* with Melanin as UV protectant

Time exposure (h)	Native strain *Bacillus thuringiensis* BGC-1 formulation					Reference strain HD1 formulation				
	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)
0	28.5	2.02×10^5	96.67 (79.48)a	2.83×10^8	159.94	29.0	2.04×10^5	93.33 (75.03)a	2.92×10^8	143.40
1	36.1	2.13×10^5	90.00 (71.57)a	2.68×10^8	133.11	34.2	2.06×10^5	90.00 (71.57)a	2.88×10^8	138.35
2	38.2	3.02×10^5	90.00 (71.57)a	2.42×10^8	124.45	37.4	3.00×10^5	90.00 (71.57)a	2.63×10^8	126.49
3	39.1	3.70×10^5	86.67 (68.58)b	2.23×10^8	120.77	38.9	3.50×10^5	86.67 (68.58)a	2.32×10^8	115.28
4	41.4	4.69×10^5	86.67 (68.58)b	2.19×10^8	118.78	42.0	4.52×10^5	86.67 (68.58)a	$2.13.\times10^8$	114.58
5	43.6	4.93×10^5	86.67 (6.58)b	1.89×10^8	116.49	43.5	4.86×10^5	86.67 (68.58)a	2.01×10^8	113.97
S. Em ±			2.22	0.07	2.49			1.99	0.08	2.42
CD @ 1%			9.61	0.30	10.50			8.62	0.35	10.46

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

Table 5. Effect of sunlight and temperature on larvicidal activity, colony count and crude protein of native *Bacillus thuringiensis* strain BGC-1 and reference strain HD1 formulation against *Helicoverpa armigera* with Congo red as UV protectant

Time exposure (h)	Native strain *Bacillus thuringiensis* BGC-1 formulation					Reference strain HD1 formulation				
	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)	Temperature (°C)	Light Intensity (lux)	Per cent mortality at 120 h	CFU/ml	Crude protein (µg/ml)
0	29.0	2.04×10^5	93.33 (75.03)a	2.80×10^8	159.57	26.6	1.54×10^5	96.67 (79.48)a	2.87×10^8	135.78
1	34.2	2.06×10^5	86.67 (68.58)ab	2.68×10^8	120.43	28.9	1.96×10^5	83.33 (65.91)b	2.63×10^8	124.42
2	37.4	3.00×10^5	80.00 (63.43)bc	2.45×10^8	115.28	31.6	2.04×10^5	83.33 (65.91)b	2.46×10^8	115.75
3	38.9	3.50×10^5	76.67 (61.11)c	2.09×10^8	107.84	35.8	2.05×10^5	80.00 (63.43)b	2.01×10^8	108.83
4	42.0	4.52×10^5	76.67 (61.11)c	$1.85.\times10^8$	105.08	36.5	2.15×10^5	76.67 (61.11)b	$1.63.\times10^8$	102.33
5	43.5	4.86×10^5	73.33 (58.91)c	1.26×10^8	90.76	41.6	4.52×10^5	73.33 (58.91)b	1.28×10^8	94.88
S. Em ±			2.22	0.07	1.71	0.06		2.48	0.08	2.42
CD @ 1%			9.61	0.30	7.19	0.26		10.72	0.35	10.46

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

One of the main disadvantages is that the insecticidal activity of *B. thuringiensis* formulation is unstable and rapidly loses its activity under field conditions due to UV radiation (Sansinenea and Ortiz, 2014). The major role of the melanin is that it confers resistance to UV light, absorbing a broad range of the electromagnetic spectrum and preventing photo induced damage. Therefore, melanin has been commercially used in photoprotective creams and eye glasses and at the same time protects several bacterial species from UV radiation (Nosanchuk and Casadevall, 2003).

To compare cost benefit ratio among different uv protectants tested, melanin was found to be more expensive than other uv protectants like para amino benzoic acid, congo red and polyvinyl alcohol. But melanin is a natural pigment, is easily biodegradable in the nature, and absorbs uv radiation consequently photo protection of Bt. Congo red is an azo dye, it is toxic and a suspected carcinogen and mutagen. Hence there is need to reduce the production cost of melanin. Therefore, melanin could be used as new UV protectants for developing Bt formulation to protect Bt toxins under field conditions.

REFERENCES

Abbott WS. 1925. A method for computing the effectiveness of an insecticide. *J Econ Entomol*. **18**: 265–267. https://doi.org/10.1093/jee/18.2.265a

Amoura M, Brayner R, Perullini M, Sicard C, Roux C, Livage J, Coradin T. 2009. Bacteria encapsulation in a magnetic sol–gel matrix. *J Mater Chem.* **19:** 1241–1244. https://doi.org/10.1039/b820433k

Ashfaq M, Young SY, McNew RW. 2001. Larval mortality and development of *Pseudoplusia includens* (Lepidoptera: Noctuidae) reared on a transgenic *Bacillus thuringiensis* cotton cultivar expressing Cry1Ac insecticidal protein. *J Econ Entomol*. **94**(5): 1053–1058. https://doi.org/10.1603/0022-0493-94.5.1053

Bernhard K, Utz R. 1993. Production of Bacillus thuringiensis insecticides for experimental and commercial uses. In: Bacillus thuringiensis, an environmental biopesticide: Theory and practice, pp. 255–267.

Brar SK, Verma M, Tyagi RD, Valero JR, 2006. Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process Biochem.* **41**: 323–342. https://doi.org/10.1016/j.procbio.2005.07.015

Burton SL, Ellar DJ, Li J, Derbyshire DJ. 1999. N Acetylgalactosamine on the putative insect receptor aminopeptidase N is recognised by a site on the domain III lectin-like fold of a *Bacillus thuringiensis* insecticidal toxin. *J Mol Biol*. **287**(2): 1011–1022. https://doi.org/10.1006/jmbi.1999.2649

Dulmage HT, Boening OP, Rehnborg CS, Hansen GD. 1971. A proposed standardized bioassay for formulations of *Bacillus thuringiensis* based on the international unit. *J Invertebr Pathol*. **18**: 240-245. https://doi.org/10.1016/0022-2011(71)90151-0

Finney DJ. 1971. *Probit analysis*. Cambridge University, Cambridge. pp. 20-49.

Goudar DG. 2011. *Isolation, characterization and development of Bacillus thuringiensis formulations against diamond back moth (Plutella xylostella L.)*. Ph. D thesis, Univ. Agric. Sci., Dharwad, Karnartaka (India).

Hadapad AB, Hire RS, Vijayalakshmi N, Dongre TK. 2009. UV protectants for the biopesticide based on *Bacillus sphaericus* Neide and their role in protecting the binary toxins from UV radiation. *J Invertebr Pathol*. **100**: 147–152. https://doi.org/10.1016/j.jip.2008.12.003 PMid:19167401

Hadpad AB, Vijayalakshmi N, Hire RS, Dongre TK. 2008. Effect of ultraviolet radiation on spore viability and mosquitocidal activity of an indigenous ISPC *Bacillus sphaericus* Neide strain. *Acta Trop*. **107**(1): 113–116. https://doi.org/10.1016/j.actatropica.2008.04.024 PMid:18538292

Justin CGL, Soudararajan RP, Rabindra RJ, Swamiappa M. 2001. Dosage and time mortality response of the *P. xylostella* (L.) to *B. thuringiensis* Berliner formulations. *Pest Manage Ecol Zool.* **9**(1): 109–113.

Kashyap S, Amla DV. 2007. Characterisation of *Bacillus thuringiensis kurstaki* strains by toxicity, plasmid profiles and numerical analysis of their CryIA genes. *African J. Biotechnol*. **6**(2): 1821-1827.

Kranti KR. 2005. Insecticidal resistance management in cotton to enhance productivity. Model training course on cultivation of long staple cotton. Central Institute for Cotton Research, Regional Station, Coimbatore, Dec 15-22: 214-231.

Lakshminarayana M, Sujatha M. 2003. Efficacy of *Bacillus thuringiensis* proteins against the lepidopteran pest complex of castor. Proceedings of the National Symposium on Frontier Areas of Entomological Research, 5-7 November. pp. 459–460.

Lalitha C, Muralikrishna T, Sravani S, Devaki K. 2012. Laboratory evaluation of native *Bacillus thuringiensis* isolates against second and third instar *Helicoverpa armigera* (Hubner) larvae. *J Biopest*. **5**(1): 4–9.

Lowry OH, Rosebrough NJ, Lewis Farr A, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem*. 193: 265–275. PMid:14907713

Magda A, Bendary EI. 2006. *Bacillus thuringiensis* and *Bacillus sphaericus* biopesticides production. *J Basic Microbiol.* **46**: 158–170. https://doi.org/10.1002/jobm.200510585 PMid:16598830

Malik K, Jabeen F, Talpur MMA, Andleeb S, Farooq A. 2013. Pesticidal activity of Pakistani *Bacillus thuringiensis* isolates against *Helicoverpa armigera* (Hubner) and *Earias vittella* (Lepidoptera: Noctuidae). *J Pharmacy and Biol Sci*. **4**(1): 9–12. https://doi.org/10.9790/3008-0460912

Navon A, Klein M, Braun S. 1990. *Bacillus thuringiensis* potency bioassay against *Heliothis armigera, Earies insulana* and *Spodoptera littoralis* larvae based on standard diets. *J Invertebr Pathol*. **55**(1): 387–393. https://doi.org/10.1016/0022-2011(90)90082-H

Nosanchuk JD, Casadevall A. 2003. The contribution of melanin to microbial pathogenesis. *Cell Microbiol*. **5**(1): 203–223. https://doi.org/10.1046/j.1462-5814.2003.00268.x PMid:12675679

Praca LB, Caixeta CF, Gomes ACM, Monnerat RG. 2013. Selection of Brazilian *Bacillus thuringiensis* strains for controlling diamondback moth on cabbage in a systemic way. *Bt Res.* **4**(1): 1–7.

Ragesh PR, Satish G, Singh IK, Singh AK. 2015. Attraction of neonate *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) larvae to different host plant volatiles. *J Entomol Zool Studies* **3**(3): 94–97.

Sansinenea E, Ortiz A. 2014. Melanin: A photoprotection for *Bacillus thuringiensis* based biopesticide. Springer, Mexico. *Biochem Pharmacol*. **3**(3): 1–8. https://doi.org/10.4172/2167-0501.1000e161

Savitri G, Muralimohan P. 2003. Pathogenicity of the bacterium *Bacillus thuringiensis* coagulans in silkworm, *Bombyx mori* (Linneaus). *Indian J Seric*. **42**(1): 4–8.

Sharma JP, Reddy AM. 1993. Studies on toxicity of some biopesticides against *Helicoverpa armigera* (Hub.). *J Insect Sci*. **6**(2): 292–294.

Sharma SS, Kaushik HD, Kalra VK. 2014. Toxicity of *Bacillus thuringiensis* varieties *kurstaki* and *aizawai* against some lepidopterous pests. *Ann Biol*. **17**(1): 91–94.

Vimala Devi PS, Vineela V. 2014. Suspension concentrate formulation of *Bacillus thuringiensis* var. *kurstaki* for effective management of *Helicoverpa armigera* on sunflower (Helianthus annuus). *Biocontrol Sci Techn.* **25**(3): 329–336.

Yang X, Oluwafemi AR, Zhang H. 2007. Screening of highly toxic *Bacillus thuringiensis* and its effects on the growth and development of *Spodoptera exigua* (Lepidoptera: Noctuidae). *Entomology General*. **31**(1): 95–104. https://doi.org/10.1127/entom.gen/31/2008/95

Yates FY. 1937. *The design and analysis of factorial experiments*. Common Wealth Bureau of Soil Science and Technology Community. pp. 35.

Zaz GM. 1989. Effectiveness of *Bacillus thuringiensis* Berliner against different instars of Spodoptera litura. *Indian J Pl Prot*. **17**(1): 119–121.

Zhang L, Zhang X, Zhang Y, Wu S, Gelbic I, Xu L, Guan X. 2016. A new formulation of *Bacillus thuringiensis*: UV protection and sustained release mosquito larvae studies. *Nature* **6**: 39425.