The Andhra Agric. J 59 (2):323-326, 2012

Effect of Temperature, Light and pH on Growth of *Bacillus thuringiensis* Ber.

Key words : *Bacillus thuringiensis* Ber., Growth, Light, pH, Temperature,

Bacillus thuringiensis (*Bt*) is an endospore forming gram positive soil bacterium that upon sporulation produces crystalline proteinaceous inclusions that are toxic mainly to lepidopteran, dipteran and coleopteran pests. However, one of the main disadvantages with the use of *Bt* is that its formulations are inadequately stable under field conditions and rapidly loose their activity (Ignoffo *et a*l., 1974). Inactivation of *Bt* formulations and its spores by solar radiation is considered as the major environmental factor affecting the efficacy (Morris, 1977). Other factors such as temperature and pH also play a role affecting the efficacy of *Bt* (Lacey and Federici, 1979).

The laboratory investigation was conducted during 1999-2000 with a commercial formulation of *Btk* (Delfin WG, strain: SA 11 3a 3b, M/s Margo Biocontrol Pvt. Ltd., Tumkur, India) and its pure culture obtained by inoculating the formulation on nutrient agar medium were used in the experiment. The median effective concentrations (EC_{50}) of *Btk,* both as a commercial formulation and pure culture (0.16% and 1.8 x 10^5 cells/ml, respectively), calculated against third instar larvae of *Spodoptera litura* (Fab.) in response to 50 per cent pupation (Srinivasan *et al*., 2001) were used as test concentrations.

Effect of Temperature:

Effect of different temperatures *viz*., 10^0C, 20^0C, 30^0C and 40^0C on growth of *B.t.* both from the formulation and pure culture was tested by inoculating *Bt* at its EC_{50} (0.16% formulation) and (1.8 x 10^5 cells/ml pure culture) on to nutrient agar medium of neutral pH in petriplates in a Biological Oxygen Demand (BOD) incubator set at the required temperature.

After incubation for 24 h the bacterial colonies developed on the agar plates were harvested aseptically as stock suspension using 10 ml sterile water. The stock suspension was serially diluted by transferring 1 ml of suspension successively into a series of sterile water blanks to obtain dilutions of 10:1, 10:2 till 10:7. The concentration of bacterial cells in each of the dilutions and that of the stock suspensions were estimated by plate count method (Fox, 1996). One ml of the suspension from each of the dilutions as well as the stock suspension was aseptically inoculated on to the agar plates. After incubation for 24 h the colonies were counted with the help of Quebic colony counter. Average of 3 replications gave the number of colonies per each dilution.

The concentration of bacterial cells in respect of each dilution as well as suspension was estimated using the formula,

$$\text{No. of cells/ml} = \frac{\text{No. of cells in the dilution} \times \text{Dilution factor}}{\text{1ml of suspension}}$$

Effect of Light:

Effect of light of different wavelengths at different intensities (UV, Blue, Yellow and Red at 450, 600 and 750 1x and sunlight at 6500, 7000 and 7500 1x) on growth and development of *Bt* was studied. The source of visible light was a 100 W tungsten bulb installed in a wooden chamber covered in and out with perforated white papers. The different coloured visible lightings were obtained by covering the bulb with heat resistant cellophane colour filters. The different intensities of light was measured using digital lux meter. Study with UV light was conducted in a laminar airflow chamber wherein the source of UV light was 100 W germicidal lamp. The effect of sunlight was studied by exposing the treatments to different sunlight intensities like direct sunlight and sunlight under shade.

The EC_{50} solutions of the commercial formulation and pure culture were inoculated on nutrient agar medium at neutral pH in petriplates, and were exposed to lights of different wavelengths and intensities for 24 h. There after the bacterial colonies developed on the agar plates were harvested aseptically using 10 ml of sterile distilled water. The bacterial suspension prepared was

Table 1. Effect of temperature on the growth and development of *Bt* as formulation and pure culture

S. No.	Temperature (^{0}C)	Number of *B.t* cells/ml x 10^7	
		Formulation	Pure Culture
1	10	0.07^c	0.08^c
2	20	4.80^b	5.20^b
3	30	7.30^a	7.00^a
4	40	6.90^a	6.60^a
F-test		Sig.	Sig.
SEd		0.31	0.50
C.D.		0.72	1.16

In each column, means with similar alphabets do not vary significantly at P=0.05 by Duncan's multiple range test.

serially diluted and the concentration of bacterial cells in each of the dilutions as well as in the stock suspension was estimated.

Effect of pH:

Nutrient agar media with different pH were tested for growth of *Bt* Media were prepared with different pH values viz., 6, 7, 8, 9, 10 and 11 by adding sufficient quantities of 1N NaOH and 1N HCL drop by drop (Behle *et al*., 1997). The pH levels of the media were confirmed using pH meter. Then the *Bt* solutions at its EC_{50} both as a formulation (0.16%) and pure culture (1.8 x 10^5 cells/ml) were inoculated on the media with different pH. After inoculated for 24 h the bacterial colonies developed on the agar plates were harvested and estimated.

Effect of temperature

The different temperatures tested *viz.*, 10^0C, 20^0C, 30^0C and 40^0C significantly affected the growth and development of *Bt* on nutrient agar medium inoculated at its EC_{50} either as the commercial formulation or as a pure culture. The mean number of *Bt* cell/ml of bacterial suspension harvested was highest at 30^0C both from the formulation (7.3 x 10^7) and pure culture (7.00 x 10^7). The populations of *Bt.* cells/ml harvested at different temperatures are 6.9 x 10^7, 4.80 x 10^7 and 0.07 x 10^7 from formulation and 6.60 x 10^7, 5.20 x 10^7 and 0.08 x 10^7 from pure culture at 40^0C, 20^0C and 10^0C, respectively (Table 1). This differential effect was attributed to various degrees of bacterial growth at various temperatures. In our present investigations *Bt* at constant dose when subjected to different temperatures both as formulation and pure culture for 24 h resulted in increased number of cells with increase in temperature.

Effect of light

There was no significant effect of different intensities of visible light tested on the population growth of *Bt* cells on the medium either from formulation or pure culture. Among the visible spectra tested, yellow light at 750 1x was superior in resulting the highest number of *Bt* cells/ml. However, there was 90.94 to 91.62 per cent reduction in the growth of *Bt* when irradiated with UV light resulting only 6.2 x 10^6 to 6.7 x 10^6 cells/ml. Exposure to sunlight resulted in 51.35 to 58.11 per cent reduction in the growth of *Bt* (3.10 x 10^7 to 3.60 x 10^7 cells/ml) (Table 2).

The present findings of effect of different wavelengths of visible light (Blue, Yellow and Red) are in agreement with those of Morris (1983), who reported that visible light of 450-700 nm did not effect the growth of *Bt.* The present investigation suggested that exposure of the bacterium to UV light resulted in 90.94 to 91.62 per cent loss of its viability compared to visible light, indicating the germicidal effect of UV light. These results are in conformation with the earlier findings of Ignoffo and Garcia (1978), who reported 95.00 per cent inactivation of *Bt* on exposure to UV light, respectively. This inactivation of *Bt* spores could be due to the peroxide or peroxide radicals produced by UV radiation of amino acids, which results in the destruction of tryptophan and histidine residues.

Table 2. Effect of light on the growth of *Bt* as formulation and pure culture

S. No.	Type and Intensity of light		Number of *Bt* cells/ml x 107	
			Formulation	Pure culture
1	Red	450 1x	6.60[a]	6.50[a]
2		600 1x	6.80[a]	7.30[a]
		750 1x	7.10[a]	7.00[a]
4	Blue	450 1x	6.80[a]	6.70[a]
5		600 1x	7.20[a]	7.30[a]
6		750 1x	7.20[a]	7.40[a]
7	Yellow	450 1x	6.70[a]	6.10[a]
8		600 1x	6.90[a]	6.40[a]
9		750 1x	7.40[a]	7.20[a]
10	UV	450 1x	0.62[b]	0.64[b]
11		600 1x	0.64[b]	0.67[b]
12		750 1x	0.63[b]	0.65[b]
13	Sunlight	6,5001x	3.40[c]	3.60[c]
14		7,000 1x	3.20[c]	3.40[c]
15		7,500 1x	3.10[c]	3.20[c]
F-test			Sig.	Sig.
SEd			0.41	0.44
C.D.			0.84	0.89

In each column, means with similar alphabets do not vary significantly at P=0.05 by Duncan's multiple range test.

Table 3. Effect of pH on the growth and development of *Bt* as formulation and pure culture

S. No.	pH	Number of *Bt* cells/ml x 107	
		Formulation	Pure culture
1	6	0.75[e]	0.89[e]
2	7	6.90[d]	7.40[d]
3	8	8.10[ab]	8.20[cd]
4	9	8.50[a]	8.80[ab]
5	10	8.70[a]	9.10[a]
6	11	7.60[c]	8.00[bc]
F-test		Sig.	Sig.
SEd		0.25	0.29
C.D.		0.55	0.65

In each column, means with similar alphabets do not vary significantly at p=0.05 by Duncan's multiple range test.

Results of the present study indicated that exposure to sunlight of different intensities resulted in 51.35 to 58.19 per cent loss of viability of *Bt* spores. These results are in accordance with those of Poszgay *et al*. (1987), who reported 35 per cent inactivation of *Bt* spores on exposure to sunlight respectively. It was assumed that the effect of sunlight on *Bt* was due to the UV portion of the sunlight or in part due to the wavelengths of sunlight near 400-420 nm.

Further, it was also suggested a photo inactivation mechanism by which an exogenous chromospore, presently unidentified and non-covalently bound to the crystal, absorbs light and transfers energy to O_2 molecule. This in turn creates a sing-let oxygen, which would react with the tryptophan and histidine residues. The destruction of tryptophan residues may result in profound changes in the configuration of the toxic protein and consequently results in loss of its biological activity.

Effect of pH

There was significant difference in the growth of *Bt* grown at different pH levels of the medium tested either from the formulation or pure culture. The mean number of *Bt* cells/ml harvested was highest at pH 10 with 8.70 x 10^7 cells from formulation and 9.10 x 10^7 cells from pure culture. The descending order of *Bt* growth at other pH levels *viz*., 9, 8, 11, 7 and 6 with the mean number of cells/ml was 8.50 x 10^7, 8.10 x 10^7, 7.60 x 10^7, 6.90 x 10^7 and 0.75 x 10^7 from the formulation and 8.80 x 10^7, 8.20 x 10^7, 8.00 x 10^7, 7.40 x 10^7 and 0.89 x 10^7 from pure culture, respectively (Table 3). The growth of *Bt* in terms of number of cells/ml of bacterial suspension at different pH levels *viz*., 6, 7, 8, 9, 10 and 11 was significantly different. The mean number of *Bt* cells/ml was highest at pH 10 (8.70 x 10^7 and 9.10 x 10^7) and lowest at pH 6 (7.50 x 10^6 and 0.89 x 10^7) both from the formulation and pure culture. These results are in conformation with those obtained earlier by Saleh *et al.* (1970) who reported 10 fold reduction in growth of *Bt* at pH 6. The protoxin in the spore coat is responsible for the alkaline activation of *Bt* (Wilson and Benoit, 1993). Hence, dissolution of the protoxin from the coat could activate the spore by increasing porosity and thereby exposing the germination trigger.

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(Received on 20.06.2011 and revised on 02.08.2011)