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THE INFLUENCE OF MOISTURE AND TEMPERATURE ON THE SURVIVAL OF *BACILLUS THURINGIENSIS* BERLINER IN AUTOCLAVED SOIL

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ABSTRACT

Experiments were conducted to investigate the influence of moisture and temperature on the survival of two isolates (B.t. -16 and B.t. - 64) of *Bacillus thuringiensis* Berliner in autoclaved soil. Both isolates under different moisture and temperature regimes exhibited decreasing trend in populations with time – mostly during first week of observation. Population of surviving bacteria related to moisture and temperature in curvilinear fashion. There were relatively more mortalities under extreme cold, hot, wet or dry conditions. The survival of the isolates was extremely low but still of the size of $\log_{10} 7$ units. The survivorship indicated that B.t.-64 was relatively more tolerant to moisture austerity and temperature extremes than isolate B.t.-16.

Key words: *Bacillus thuringiensis*, survival, moisture levels, temperature.

INTRODUCTION

Bacillus thuringiensis Berliner is a rod shape, gram positive, aerobic, spore forming opportunistic bacterium. It is everywhere on the planet, deserts, beaches and tundra (Martin and Travers, 1989). Ishiwata first discovered this bacterium in 1901 in Japan from diseased silkworm larvae, identified as sotto disease of silkworm. A German biologist Berliner (1911) isolated it from pupae of Mediterranean flour moth *Ephastia kuchmiella* living in stored grains in the city of Thuringen and hence given name as *B. thuringiensis* (B.t.). It is a potent biopesticide (Baucias and Pendland, 1988) and a versatile pathogen. It produces toxins on sporulation which is active against more than 130 insects (Dean, 1984). It has been used as a biological control agent for long time and is an effective measure for the control of a wide range of insect pests (Trailer *et al.,* 1992; Borgonie *et al*., 1996; Nester et *al.,* 2002; Wei *et al*., 2003). It is capable of infecting protozoa, nematodes, flatworms, mites and insects (Feitelson, 1993). It produces parasporal crystals of protein known as delta-endotoxins that are toxic to insect pests (Höfte & Whitefley, 1989). This unique property of producing delta-endotoxins distinguished *B. thuringiensis* from other spore forming bacteria. Spores are the dormant stage of their life cycle, which germinate when the conditions are favorable for their growth. The protein crystal is the toxic component of *B. thuringiensis*. The toxins are inactivated within days in soil (Petras and Casida, 1985) and endospore may remain in soil for months or years (Saleh *et al.,* 1970; Petras and Casida, 1985).

B. thuringiensis is moderately persistent bacterium in soil and its toxins degrade rapidly in soil (Kamrin, 1997; WHO, 1999). Kaur and Singh (2000) have investigated distribution of *B. thuringiensis* in soils of Northern India. B.t.'s population in soil of Tarakeswar, Hooghly, West Bengal, India is reported to range between 4.23×10^5 and 6.52×10^5 cfu / g soil (Chatterjee *et al.,* 2007). The survival of *B. thuringiensis* introduced into soil depends upon several associated environmental factors- soil texture, moisture, temperature, pH, nutrients, etc. Despite wide use of *B. thuringiensis*, very little is known about survival of this bacterium in soil at different moisture and temperature levels (Petras and Casida Jr, 1985; West and Burges, 1985). Introduction of laboratory-grown bacterial cultures into soil is a common practice to study microbial growth. Present studies have been conducted to investigate the impact of different levels of moisture and temperature on population of two B.t. isolates (B.t. 16 and B.t. 64) in autoclaved soil. These are two very important isolates amongst the 11 isolates obtained in Pathological Laboratory of Karachi University, Karachi and their characterization is being published elsewhere.

MATERIALS AND METHODS

The soil employed in this experiment was collected from the Experimental Field of the Department of Botany, University of Karachi, Karachi. Soil was thoroughly mixed, air-dried and sieved through a fine mesh and stored for further studies. The soil was sandy loam in texture, non-saline ($0.50\ dS.m^{-1}$) basic in reaction (pH: 7.5 – 8.1). The percent Maximum water holding capacity of the soil as determined by the method of Keen and Raczkowski (1921) was 40 and nitrogen content as ascertained by the method of Mackenzie and Wallace (1954) was 0.08-1.0%. The organic content of the soil was c 1.0%.

The Petri plates were filled with 25g of the soil and were autoclaved at 121ºC at 15 psi for 15 minutes. Thereafter, in each Petri plate one mL of bacterial suspension was poured. One mL of bacterial suspension of B.t.-16

and B.t.-64, introduced in the soil in our experiment related to moisture, contained 2.225358578 x 10^9 and 8.27560930 x 10^8 cells, respectively. Moisture level (0, 25, 50, 75 and 100% of Maximum Water Holding Capacity of the soil (MWHC)) was maintained by adding calculated amount of sterile water. Each treatment was replicated thrice. To maintain soil moisture level, the Petri plates were weighed daily and sterilized distilled water was added to maintain the initial weight. The observations were made after 0, 7, 14, 21 and 28 days by taking 1g soil from each replicate and preparing a serial dilution. 1mL bacterial suspension from the appropriate dilution was poured into Petri plates, and autoclaved and cool nutrient agar was poured in each Petri plate. The plates were then incubated at 37° C for 24 h and bacterial count was made at proper times.

To determine the survival of *B. thuringiensis* isolates in soil at different temperatures, Petri plates were filled with 25 g soil and were autoclaved at 121 ^oC at 15 psi for 15 minutes. Then, one mL of bacterial suspension was poured in each Petri plate. One mL of bacterial suspension introduced in the soil contained 1.392515378 x 10^{10} B.t.-16 cells and in case of B.t.-64, 6.00073539 x 10^8 cells. Soil in each Petri plate was maintained at field capacity (c 50% moisture holding capacity). Petri plates were incubated at different temperatures (-10, 10, 20, 30 and 50°C). To maintained soil moisture level the Petri plates were weighed daily and calculated amount of sterilized distilled water was added to maintain moisture. Each treatment was replicated thrice. The observations were taken after 0, 7, 14, 21 and 28 days by taking one g soil from each replicate and making a serial dilution. 1mL bacterial suspension from the appropriate dilution was poured into Petri plates, and autoclaved and cool nutrient agar was poured in each Petri plate. The plates were then incubated at 37° C for 24 h and bacterial count was made.

RESULTS

The response of the populations of B.t.-16 and B.t.-64 to various moisture regimes over a period of 28 days is portrayed in surface plot (Fig. 1 and 2). The population size of the surviving bacteria of the two isolates (log cfu / g soil) behaved curvilinearly to the moisture availability. After 7 days, there was maximum survival of the bacteria in moisture level of 50% MWHC, on either side of which, the surviving population declined significantly. The population of the culturable bacteria declined very rapidly during the first week of incubation – subsequently the decline was slow but most of the population died after 28 days. The population decline was around 2 $\log_{10}$ units from $\log_{10}$ units 9 to $\log_{10}$ units 7 in B.t.-16 and 1 $\log_{10}$ unit from $\log_{10}$ units 8 to $\log_{10}$ units 7 in case of B.t. - 64. The two-way ANOVA of the data indicated that the two factors, time and moisture, in either case influenced the populations significantly and they interacted significantly with each other also (Table 1 and 2). The populations of the two isolates in the given domain of moisture and time were defined by the following equations.

B.t. -16

$$\text{Log}_{10}\text{ CFU/g soil} = 8.982 - 0.118\ (\text{time}) + 0.02\ (\text{moisture}) + 0.002\ (\text{time})^2 - 0.00008998\ (\text{moisture x time}) - 0.0001975\ (\text{moisture})^2$$

B.t. - 64

$$\text{Log}_{10}\text{ CFU/g soil} = 8.612 - 0.072\ (\text{time}) + 0.021\ (\text{moisture}) + 0.002\ (\text{time})^2 - 0.0001553\ (\text{moisture x time}) - 0.0002105\ (\text{moisture})^2$$

The behaviour of the bacterial populations of isolates B.t.-16 and B.t-64 against temperature profile over a period of 28 days is depicted as surface plots in Fig. 3 and 4. The responses of the two populations (log cfu / g soil) were essentially similar – the survival of both isolates in response to temperature was curvilinear. The survival was the maximum at 30 ^oC – on either side of this temperature surviving population declined significantly as compared to that at 30^oC. There was sharp reduction of populations in the early period of incubation. After 28 days, the population of B.t. -16 declined 3 $\log_{10}$ units from $\log_{10}$ units 10 to $\log_{10}$ units 7. At the mid temperature range, the decline in isolate B.t.- 64 was from $\log_{10}$ units 9 to $\log_{10}$ units 8 but at the extreme temperature of -10 and 50 ^oC the decrease was of 2 $\log_{10}$ units. The Two-way ANOVA of the data indicated significant effects ($p < 0.0001$) of time and temperature over populations of both isolates. The two factors interacted significantly (Table 3 and 4). The overall effects of time and temperature over populations emerged as curved sheets and were given by the following equations.

B.t. -16:

$$\text{Log}_{10}\text{ CFU/g soil} = 10.042 - 0.103\ (\text{time}) + 0.04\ (\text{temperature}) + 0.0005656\ (\text{time})^2 - 0.00008\ (\text{temperature x time}) - 0.0009633\ (\text{temperature})^2$$

B.t. - 64

$$\text{Log}_{10}\text{ CFU/g soil} = 9.731 - 0.096\ (\text{time}) + 0.043\ (\text{temperature}) + 0.0010(\text{time})^2 - 0.0001349\ (\text{temperature x time}) - 0.001\ (\text{temperature})^2$$

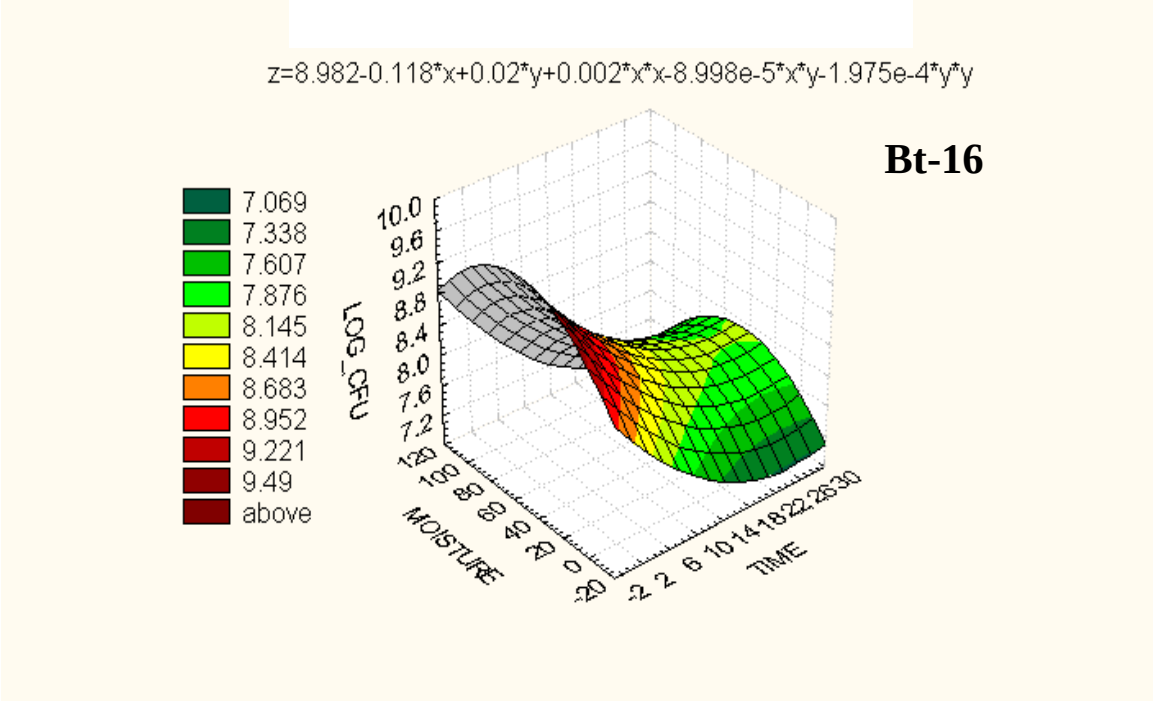

Fig. 1. Survival of BT-16 population in soil with varying moisture and time of incubation.
(X =Time: 0 -28 days); Y = Moisture, % MWHC: 0 - 100) and Z = Population, $\log_{10}$ cfu/ g Soil).

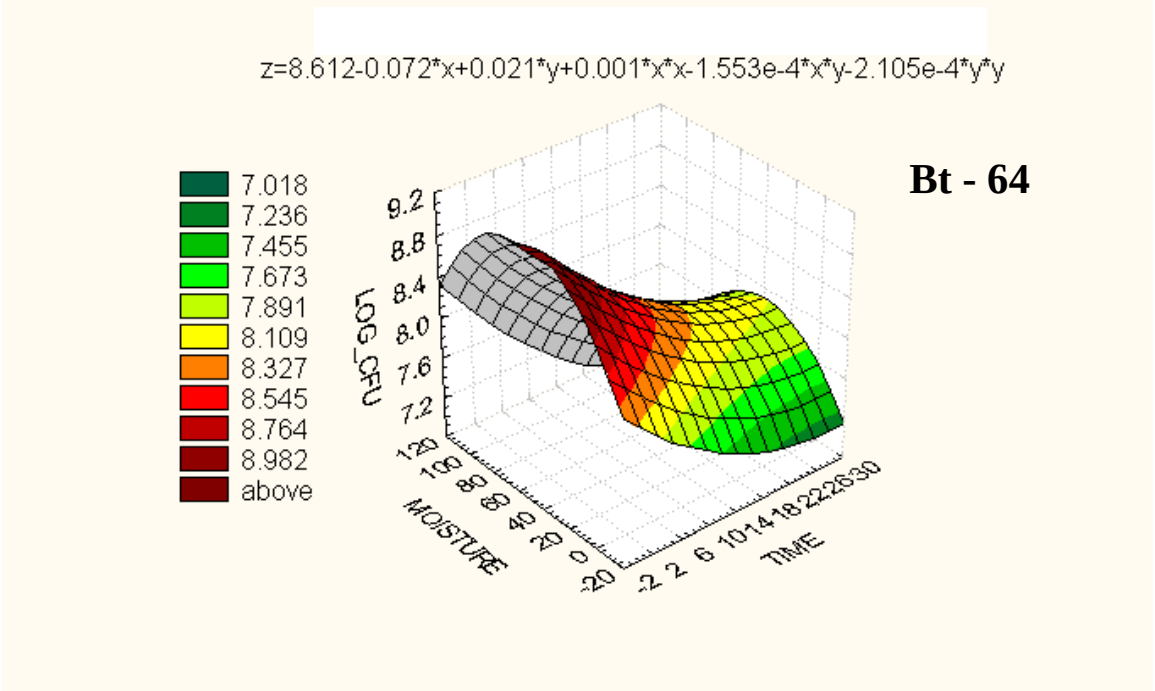

Fig. 2. Survival of BT-64 population in soil with varying moisture and time of incubation.
(X =Time: 0 -28 days) ; Y = Moisture, % MWHC: 0 - 100) and Z = Population , $\log_{10}$ cfu / g Soil).

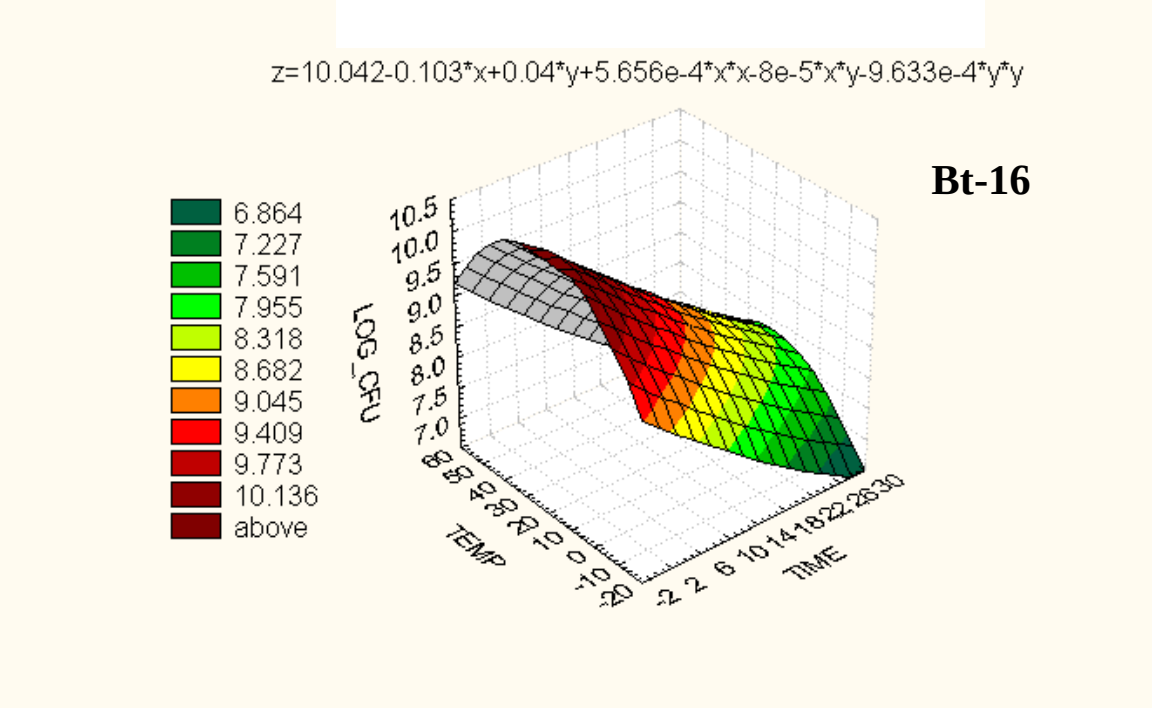

Figure 3. Survival of Bt-16 population in soil with varying temperature and incubation period. X= Time in days (0-28 days); Y= Temperature (^{o}C; -10 to 50) and Z = Population, $\log_{10}$ cfu /g SOIL).

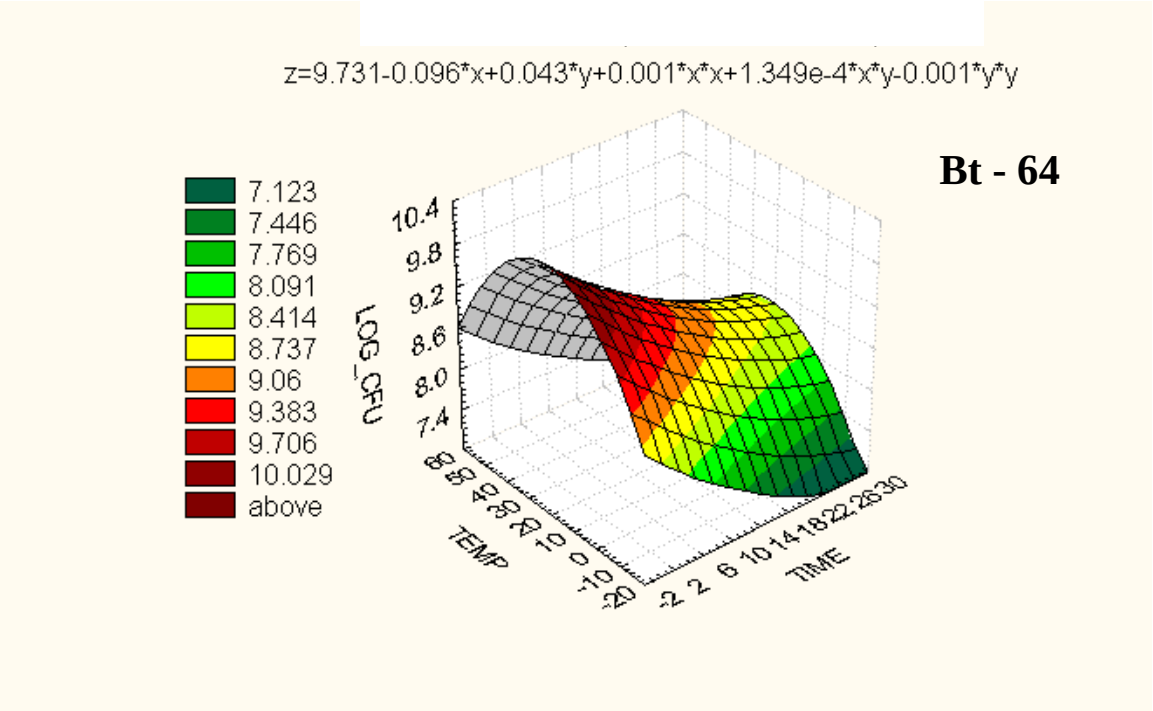

Figure 4. Survival of Bt-64 population in soil with varying temperature and incubation period. X= Time in days (0-28 days); Y= Temperature (^{o}C; -10 to 50) and Z = Population, $\log_{10}$ cfu /g SOIL).

Table 1.Two- way ANOVA for complete randomized design of interaction of moisture and time of incubation on survival of B.t. 16 in soil.

Source	SS	df	MS	F	p
Time (T)	28.4792	4	7.11982	219.129	0.0001
Moisture (M)	3.73622	4	0.93405	28.7477	0.0001
T x M	1.10636	16	0.069147	2.12817	0.05
Error	1.62457	50	0.032491	-	-
Total	34.9464	74	-	-	-
Time LSD $_{0.05}$: 0.13220; Moisture LSD $_{0.05}$: 0.13220					

Table 2. Two- way ANOVA for complete randomized design of interaction of moisture and time of incubation on survival of B.t. 64 in autoclaved soil.

Source	SS	df	MS	F	p
Time (T)	15.90669	4	3.97667	1044.760	0.0001
Moisture (M)	4.59185	4	1.14796	301.5953	0.0001
T x M	1.65356	16	0.10335	27.1517	0.0001
Error	0.19062	50	0.003806	-	
Total	22.3424	74			
Time LSD $_{0.05}$: 0.0.04525; Moisture LSD $_{0.05}$: 0.045248					

Table 3. Two- way ANOVA for complete randomized design of interaction of temperature and time of incubation on survival of B.t. 16 in autoclaved soil.

Source	SS	df	MS	F	p
Time (T)	59.15068	4	14.78767	1400.379	0.0001
Temperature (t)	12.45570	4	3.11392	294.886	0.0001
T x t	5.28211	16	0.03301	31.263	0.0001
Error	0.527987	50	0.010559	-	-
Total	77.4164	74			
Time LSD $_{0.05}$: 0.075366; Moisture LSD $_{0.05}$: 0.075366					

Table 4. Two- way ANOVA for complete randomized design of interaction of temperature and time of incubation on survival of B.t. 64 in autoclaved soil.

Source	SS	df	MS	F	p
Time (T)	29.43170	4	7.35792	825.3726	0.0001
Temperature (t)	15.15397	4	3.78849	424.9728	0.0001
T x t	4.21731	16	0.26358	29.5672	0.0001
Error	0.44573	50	0.008915	-	-
Total	49.2487	74	-	-	-
Time LSD$_{0.05}$: 0.06924; Temperature LSD $_{0.05}$: 0.069247					

Table 5 and 6 represents *per cent* reduction in bacterial populations over initial populations quantified on the basis of original population data under various moisture and temperature treatments. It is evident that in both isolates the populations dwindled exponentially within the first week of incubation under moisture or temperature treatment. This decline was low at 50% MWHC (79.05 and 45.26 % in B.t.-16 and B.t.- 64, respectively). After 28 days, most part of the populations (95.57 – 99.29 %) died and only a small fraction of culturable bacterial cells were detected. The decline in B.t.-64, after 28 days, at 50% MWHC was, however, only 75.92% over the initial population in spite of the fact that 97.98% of population was observed to disappear one week earlier i.e., on 21 day of incubation (Table 5). Extreme temperature of -10 and 50 ^{o}C eradicated > 95 % of the populations of both isolates in the first week. The populations in the mid temperature region dwindled not more than 65% in either isolates. At 30^{o}C, strikingly there was only 5.90% decrease in population of B.t.-64 in initial week – later the mortality increased in tremendous proportion and reached to 94.07% after 28 days. It is obvious that survival of the rate was extremely low but still of the 7 log units size. It is obvious from the survivorship of the two isolates (Table 7) that B.t.-64 is relatively more tolerant to moisture austerity and temperature extremes than isolate B.t.-16.

Table 5. Effect of moisture and incubation period on the average survival in terms of original population counts of B.t.-16 and B.t.- 64 in autoclaved oil.

Days	**B.t. 16** Moisture Levels (% of Maximum Water Holding Capacity)				
	0	25	50	75	100
0 (initial)	2.225358578 x 10^9	2.225358578 x 10^9	2.225358578 x 10^9	2.225358578 x 10^9	2.225358578 x 10^9
7	92832490.11 (-95.83) *	313256434.20 (-85.92)	466229769 (-79.05)	231953003.3 (-89.58)	122715663.50 (94.45)
14	53125129.29 (-97.61)	2.194625514 x10^8 (-90.13)	269153480.40 (-87.91)	100000000 (-95.51)	73063420.49 (-96.72)
21	33014140.86 (-98.52)	104833479.40 (-95.29)	175428439.40 (-92.12)	45793095.32 (-97.94)	29998531.81 (-98.65)
28	199988618.70 (-99.10)	72677503.38 (-96.73)	98650661.10 (-95.57)	35563131.86 (-98.40)	15870843.17 (-99.29)

Days	**B.t.-64** Moisture Levels (% of Maximum Water Holding Capacity)				
	0	25	50	75	100
0 (Initial)	8.27560970 x10^8	8.27560970 x10^8	8.27560970 x10^8	8.27560970 x10^8	8.27560970 x10^8
7	113240036.3 (-86.32)	289201139.60 (-65.05)	453001875.40 (-45.26)	199296650.60 (-75.92)	139027271.60 (-83.20)
14	73181279.77 (-91.16)	153003018.90 (-81.51)	358839299.30 (-56.64)	96538378.51 (-88.33)	40003685.10 (-95.17)
21	40003685.10 (-95.17)	92832490.11 (-88.78)	17225582.30 (-97.98)	49991941.10 (-93.96)	22289483.25 (-97.31)
28	25905978.45 (-96.87)	56454686.49 (-93.18)	199296650.60 (-75.92)	36643757.46 (-95.57)	10000000 (-98.79)

*, Figures in parenthesis denote the percent reduction in population over the initial population.

DISCUSSION

In the present studies, survival of *B. thuringiensis* isolates B.t. 16 and B.t. 64 was investigated in autoclaved soil of Karachi University campus, Pakistan, at different moisture and temperatures levels. Our results have shown exponential decrease in population counts in both bacterial isolates during first week of exposure in soil at all moisture levels. However, more rapid decrease in bacterial count was observed at low and high moisture levels. At low moisture level, decrease in bacterial population may be due to less availability of moisture for various metabolic activities of vegetative cells, cessation of cell division, cellular deterioration and spore formation to survive for long time in soil in desiccated conditions (Saleh *et al.,* 1970). West *et al.* (1984) have reported exponential decrease, in *B. thuringiensis* var. *aizawai* in natural soil of pH 5 and moisture tension of -0.10 MPa, far greater than autoclaved soil due to accelerated rate of mortality in the presence of other micro-organisms. In our studies, in general a 2 $\log_{10}$ unit decrease was observed during first week of incubation which slowed down afterwards. The decrease of 2 log units immediately after inoculation and additional 2 log units after 24 h incubation (but stabilizing over the following days corresponding to spore formation) has been reported by Ferreira *et al.* (2003). Saleh *et al.* (1970) have also reported decline of B. *thuringiensis* in soil. *B. thuringiensis* exhibited greater mortality in higher moisture levels which may be expected with an aerobic bacterium. Villas-Bôas *et al.* (2000) have demonstrated decrease in viable cells of *B. thuringiensis* var. *kurstaki* KTO(pH73-EM^R) from 4.0 x10^8 to 8.3 x 10^7 in monoculture in sterilized soil. Chatterjee *et al.* (2007) have also reported similar results. The population decline of both isolates in the present studies at high moisture level may be accentuated due to reduced oxygen availability. Reduction in germinability of bacterial spores may also be a factor involved in the reduction of population counts. There are some reports that indicate the mortality of spores during incubation in soil in laboratory conditions (Ferreira *et al.*, 2003).

B. thuringiensis is reported to survive in extreme conditions of temperature by endospore formation. For a rise of temperature from 20 to 35 ^{o}C, Ignatenko *et al.,* (1983) have reported the doubling in the titre of spores and the

increase in thermo-resistance of *B. thuringiensis*. In our studies, due to huge mortality of the bacteria, B.t.'s populations were eradicated in great proportions but their population size never reached zero and at the end of 28 days, the size of their populations was generally of 7 log units. The survivorship of the two isolates indicated that B.t.-64 was relatively more tolerant to moisture austerity and temperature extremes than isolate B.t.-16. The increase in CFU of B.t.-64 during last week of incubation under 50% moisture regime (at 37 ^{o}C) indicated to the production of new bacterial cells. The multiplication of *B.thuringiensis* is reported in nutrient-rich soil (Saleh *et al*., 1970; West and Burges, 1985). Pedersen *et al.* (1995) have speculated such hot spots of bacterial proliferation in soil. The particles of organic matter in soil may be such hot spots even in autoclaved soil with no interference with any other microbes. The inactivation of B.t. toxin in soil within around 20 days (Ferriera *et al.*, 2003) may be one of the reason to the increase of B.t. 64 between 21-28 days of incubation. We know nothing about the autotoxicity of *B. thuringiensis* to the toxin it produces at the time of spore formation. Spore-toxin interaction needs to be investigated in detail.

Table 6. Effect of temperature and the period of incubation on the average survival in terms of original population numbers of B.t. - 16 and B.t. - 64 in autoclaved soil.

Days	**B.t.-16** Temperature (^{o}C)				
	-10	10	20	30	50
0 (Initial)	1.392515378x 10^{10}	1.392515378x 10^{10}	1.392515378x 10^{10}	1.392515378x 10^{10}	1.392515378x 10^{10}
7	205068993.6 (-98.53) *	4764309868 (-65.79)	6081350013 (-56.33)	7850548482 (-43.62)	630957344.5 (-95.47)
14	114762499.9 (-99.18)	1259795347 (-90.95)	1587084317 (-88.60)	4640876378 (-66.67)	100000000 (-99.28)
21	68391164.73 (-99.51)	124279643.8 (-99.51)	169980851.3 (-98.78)	542500321.6 (-96.10)	31622776.6 (-99.77)
28	16405897.73 (-99.88)	68675210.61 (-99.51)	67313163.27 (-99.52)	86000306.69 (-99.38)	16405897.73 (-99.88)

Days	**B.t.-64** Temperature (^{o}C)				
	-10	10	20	30	50
0 (initial)	6000673539	6000673539	6000673539	6000673539	6000673539
7	199296650.6 (-96.68)	2218707237 (-63.03)	4102985668 (-31.62)	5646768716 (-5.90)	153815464 (-97.44)
14	106267386.5 (-98.23)	519158537.1 (-91.35)	1000000000 (-83.34)	2079696687 (-65.34)	100000000 (-98.33)
21	56454686.49 (-99.91)	266256365.9 (-95.56)	794694120.6 (-86.76)	1000000000 (-83.34)	43082411.05 (-99.28)
28	19346392.74 (-99.68)	132739445.8 (-97.79)	233722218.5 (-96.11)	355631318.6 (-94.07)	27089438.30 (-99.55)

*, Figures in parenthesis denote the percent reduction in population over the initial population.

Table 7. Survivorship of B.t. isolates in sterilized soil under various moisture and temperature treatments after 28 days of incubation.

	Moisture (% MWHC)				
Isolates	0	25%	50%	75%	100%
B.t.-16	0.9	3.27	4.43	1.6	0.71
B.t.-64	3.13	6.82	24.08	4.43	1.21
Temperature (^{o}C)					
Isolates	-10	10	20	30	50
B.t.-16	0.12	0.49	0.48	0.62	0.12
B.t.-64	0.32	2.21	3.89	5.93	0.45

REFERENCES

Baucias, D.G., J.C. Pedland (1998). Principles of Insect Pathology. Kluwer Acad. Publ., Boston, USA. P. 537.

Berliner, E. 1911. Ueberdie Schlaftsuchtder Nehlmottenraupe (Ephestiakuhniella Zell) unihren Erreger *Bacillus thuringiensis* n.sp. *Zeitschrift fur angewandtes Entomol,* 21: 29-56

Borgonie, G., R.V.A.N. Driessche, F. Leyns, G. Arnaut, D.D.E. Waele and A. Coomans (1995). Germination of *Bacillus thuringiensis* spores bacteriophagous nematodes (Nematoda:Rhabdita). *J. Invertebr. Pathology.*, 65: 61-67.

Chatterjee, S.D.N., T. Bhattacharya, T. K. Dangar and G. Chandra (2007). Ecology and Diversity of *Bacillus thuringiensis* in soil environment. *Afr. J. Biotech*. 6(13): 1587 – 1591.

Dean, D.H. (1984). Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*: basic principles and prospects for genetic engineering. *Biotechnol. Genetic Eng. Rev*. 2: 341 -363.

Feitelson, J. S. (1993). The *Bacillus thuringiensis* family tree, In: *Advance engineered pesticides* (Ed). Kim.L. Marcel Dekker, Inc. NY.USA. pp 63-71.

Ferreira, L.H. P.L., M. T. Suzuki, E.N. Itano, M. A. Ono and O.M.N. Arantes (2003). Ecological aspects of *Bacillus thuringiensis* in an oxisol. *Scientia Agricola*, 60: 19-22.

Höfte, H. and H.R. Whitefley (1989). Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.*, 53: 242-255.

Ignatenko, I.U.N., Z.V. Sakharaova, M.P. Khorychev and V.V. Shevtsov (1983). Effect of temperature and aeration on *Bacillus thuringiensis* growth and sporulation. *Mikrobiologiia* 52 (5); 716-716.

Ishiwata, S. (1901). On a kind of severe flacherue (sotto disease). Dainihan Sanshi, Kaiho., 114: 1-5.

Kamrin, M.A. (1997). *Pesticide Projects: Toxicity, Environmental Impact and Fate*. CRC : Boca Raton, FL Pp. 535 -539.

Kaur, S. and A. Singh (2000). Distribution of *Bacillus thuringiensis* strains in different soil types from North India. *Indian J. Ecol*., 27: 52-60.

Keen, B. A. and Raczkowski (1921). The relation between clay content and certain physical properties of soil. *J. Agric. Sci.*, 11: 441-449.

Mackenzie, H. A. and H.S. Wallace (1954). The Kjeldahl determination of nitrogen: A critical study of digestion conditions, temperature, catalyst and oxidizing agent. *Aust. J.Chem*., 7: 55-70.

Martin, P.A.W. and R.S. Travers (1989). Worldwide abundance and distribution of *Bacillus thuringiensis* isolates. *Appl. Environ. Microbiol*., 55: 2437-2441.

Nester, E.W., L.S. Thomashow, M. Metz and M. Girdon (2002). 100 years of *Bacillus thuringiensis*, a Critical Scientific Assessment ASM/ Washington.DC

Pedersen, J.C. and P.H. Damgaard, J. Eilenberg and B.J. Hansen (1995). Dispersal of Bacillus thuringiensis var. *kurstaki* in an experimental cabbage field. Can. J. microbial. 41: 18-125.

Petras, S.F. and Casida Jr, L.E. 1985. Survival of *Bacillus thuringiensis* spores in soil *App. Environ. Microbiol*, 50: 1496-1501.

Saleh, S. M., R.F. Harris, and O.N. Allen, (1970). Fate of *Bacillus thuringiensis* in soil: effect of soil pH and organic amendment. *Can. J.Microbiol.*, 16: 677-680

Tailer, R.J., Tippett, G., Gibbs, S., Pells, D., Jurdan, L and Ely, S. 1992. Identification and characterization of a novel *Bacillus thuringiensis* toxin entomocidal to Coleopteran and Lepidopteran larvae, *Mol. Microbial*., 21: 1217.

Vilas-Boas, L.A., G. F.L.T. Vilas-Boas, H.O. Saridkis, M. V.F. Lemos B. Lereclus and O.M. N. Arantes (2000). Survival and conjugation of *Bacillus thuringiensis* in a soil microcosm. *FEMS Microbiol. Ecol*. 31: 255 – 259.

Wei, J.Z., K. Hale, L. Carta,, E. Platzer, C. Wong, S.C. Fang and R.V. Aroian (2003). *Bacillus thuringiensis* Crystal proteins that target nematodes. *PNAS.*, 100: 2760-2765.

WHO. (1999). *Bacillus thuringiensis*. *Environmental Health Criteria*, 217. Geneva, Switzerland.

West, A.W. and N.D. Burges (1985). Persistence of *Bacillus thuringiensis* and *Bacillus cereus* in soil supplemented with green manure *Plant and Soil*, 83: 389-398.

West, A.W., H.D. Burges and C.H. Wyborn (1984). Effect of incubation in natural and autoclaved soil upon potency and viability of *Bacillus thuringiensis*. *Annals Invertebrate Pathology*, 44(2): 121 – 127.

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