Polish Journal of Microbiology
2004, Vol. 53, No 3, 159–166

Enhancement of Pathogenicity of *Bacillus thuringiensis* by Gamma Rays

KHALID I. KHAN[1], RIFFAT H. JAFRI and M. AHMED

Department of Zoology, University of the Punjab, Lahore, Pakistan

Received in revised form 16 June 2004

Abstract

Studies were conducted on the enhancement of pathogenicity of *Bacillus thuringiensis* by gamma rays on various species of termites. The results showed that the 72 hrs old and 60 or 70 kr irradiated culture of *B. thuringiensis* were more pathogenic than the non-irradiated ones. 168 hrs old and 150 kr (1.5 kGy) irradiated *B. thuringiensis* caused quicker mortality rate than the non-irradiated one as it shows maximum enhancement ratio (1.32).

Key words: pathogenicity, *Bacillus thuringiensis*, gamma rays, termites

Introduction

Considerable efforts have been made in recent years to develop microbial control of insects as an alternative to chemical insecticides which are becoming an environmental hazard. Since 1960s, due to insecticidal activities of toxins, *Bacillus thuringiensis* or its preparation(s) is attractive and most widely used as biocontrol agent for various insects (Chestukhina *et al.*, 1994; Collier *et al.*, 1998; Waites *et al.*, 2001). Most recently the toxicity of a selected *B. thuringiensis* toxin to a number of geographically diverse population of tomato pinworm has been described by Seal and Leibees (2003).

Radiation is considered among the most important factor that affect the development of entomopathogens, their subsequent infection to the host, their survival and virulence within the host and to limited extent the host susceptibility (Burgerjon and Yanwiras, 1959; Aruga and Yoshitake, 1961; Smirnoff and Cantin, 1967; Bullock *et al.*, 1970; Morris, 1971).

The possibilities of using gamma rays for increasing the virulence of pathogens of insects have received much attention. Smirnoff and Cantin (1967) presented results of experiments on the influence of gamma radiation on the *in vitro* growth, of six entomopathogenic varieties of *Bacillus cereus* group. Merdan *et al.* (1975) discovered that the virulence of *B. tuviensis* was significantly increased by 100 kr of gamma rays. Faruki and Khan (2001) also reported that the pathogen, irradiation and their combination significantly affected the insects by increasing their mortality and developmental periods.

The result of various series of experiments (Smyth and Coppel, 1965; Vypijack *et al.*, 1972; Khan, 1981; Khan *et. al.*, 1985) showed that *Bacillus thuringiensis* was potential biological control agent for termites. Thus, it was felt that studies should be carried out to explore the possibility of enhancing the pathogenicity of *B. thuringiensis* by gamma rays and used against various species of termites. In the present study, a safe range of gamma rays for irradiation of *B. thuringiensis* was used. Becker (2002) has mentioned that no spore survived radiation doses of 20.6 kGy and higher.

Experimental

Materials and Methods

Insects collection and their rearing. Various species of termites such as *Heterotermes indicola* (Wasmann), *Microcerotermes championi* (Snyder) and *Bifiditermes beesoni* (Gardner) were selected for laboratory experiments. The termites *H. indicola* was

[1] Present Address: College of Pharmacy, University of the Punjab, Lahore

collected from stem and branches of *Pinus roxburghii, Morus alba*, *Dalbergia sisso, Broussinile papyrifera* and *Eriobotrya japonica*; growing at Azad Kashmir and Changa Manga forest while *M. championi*, abundantly growing at roots of *Saccharum munja* was collected only from Lahore; mostly at and near Punjab University. However, *B. beesoni* was mainly infesting fruit trees such as *Prunus communis*, *Pyrus communis* and *Prunus domestica* growing in Botanical Garden of Punjab University, New Campus and Daroghawala, Lahore. The roots of *Saccharum munja* infested with *M. championi* were kept in tins of 10 lb. capacity; each tin box contained moist sand as bed. The container was placed on a raised platform of bricks, surrounded by water: The water acted as a barrier to the ants *etc.* while the logs infesting *H. indicola* or *B. beesoni* were kept in concrete container (95×72×72 cm); having a water channel (7cm wide and 6cm deep) around the rim, to provide humidity and served a barrier to ants. All the termites were maintained in the laboratory at 22–28°C with 60–80% relative humidity.

Isolation and identification of pathogen. During the course of study, the sample of sick or dead termites were collected from the field, kept in sterile Kilner's jars (2 lbs capacity) containing moist filter papers and brought to the laboratory. These termites were surfaced sterilized by 5.25% aqueous solution of sodium hypochlorite as described by Bao and Yendol (1971). The sick or dead termites were crushed in sterile distilled water and their suspensions were prepared separately. The inoculums of suspension were directly streaked on Petri dishes containing nutrient agar medium. The dilutions of these suspension were prepared and pour plate method was also used as mentioned by Cruickshank *et al.* (1975). The Petri dishes with nutrient agar were incubated at 25–30°C up to 72 hours. Different colonies growing on media were isolated and inoculated on nutrient agar slants to obtain nutrient agar pure cultures. The smear of the culture was stained and examined for morphological characters. The original method of Gram modified by Conn (1946) was used. The spore staining was performed by method of Schaeffer and Fulton modified by Ashby (1938). Motility test for the bacteria was also performed as described by Tittsler and Sandholzer (1936). This test was aided by the addition of an indicator *i.e.* triphenyl tetrazolium chloride (TTC), as mentioned by Kelly and Fulton (1953). The bacterium was identified according to Bergey's Manual of Determinative Bacteriology (Breead *et al.*, 1972).

Two bacterial isolates were obtained from sick and a few dead nymphs of *B. beesoni* collected from Lahore, the sample "LHR-1" was isolated from termites infesting *Pyrus communis*; located at Daroghawala while sample "LHR-2" was isolated from the same species of termites infesting *Prunus domestica* located at Botanical-garden of University of the Punjab. These isolates were identified in our laboratory as bacterial strains similar to *B. cereus* except that the most of them were also showing some crystals; an important characteristic of *B. thuringiensis.* These isolates were finally confirmed as *B. thuringiensis* serotype 3a, 3b by Dr. H. De Barjac, Pastuer Institute, Paris. France.

Irradiation of pathogen. Experiments were designed to enhance the pathogenicity of *B. thuringiensis* by gamma irradiation. *Bacillus thuringiensis* (serotype 3a, 3b) cultures were grown on nutrient agar medium for 72 hrs, 96 hrs and 168 hrs at 32°C. These bacterial cultures of each ages were suspended homogenously in sterile distilled water. An amount of 2 ml of each suspension kept in a vial, was exposed to Co^{60} gamma rays. Gamma celi Model 220 (Canadian maker) at Nuclear Institute of Agriculture and Biology, Faisalabad, was used. The dose rate was 10 kr/267 seconds. The suspensions were exposed to 60 kr, 70 kr, 100 kr and 150 kr doses of gamma rays. The viable count/ml of *B. thuringiensis* was determined by pour plate method. Following irradiation, a portion of the cultures from each vial was streaked on nutrient agar slants. These slants were then incubated at 32°C and their pathogenicity was tested against *H. indicola*, *M. championi* and of *B. beesoni.* The cultures of *B. thuringiensis* grown for 72 hrs and 168 hrs were used for testing their pathogenicity. While 96 hrs old culture of *B. thuringiensis* was not used further because its preliminary results were almost similar to 72 hrs old cultures.

Method of dosing (bioassay). Force feeding bioassay of *B. thuringiensis* toxins against gypsy moth, *Lymantria dispar*; using 0.25 ml syringe was described by Lee *et al.* (1996). Various commercial preparations of *B. thuringiensis* including Thuricide HP was tested against sunflower moth (Lepidoptera: Tortnicidae) by topically applying 150 µl aliquot of each concentration to the surface of artificial diet in 29.6 ml Cups (Jyoti and Brewer, 1999). Leaf-dip bioassay of *B. thuringiensis* Cry-I type insecticidal toxin against tomato pinworm (*Keiferia lycopersicella*) was conducted in the laboratory by Seal and Leibee (2003). Collier *et al.* (1998) reported that upon ingestion by filter-feeding insect larvae, the crystal protoxin (of *B. thuringiensis)* are solubilized in alkaline larval mid gut and converted into active toxin molecules. It supports the filter-feeding procedure, adopted in the present studies. The termites of 'Test' groups (25 workers or nymphs) were allowed to feed on the double-layered filter paper duly placed in Petri dishes (75mm) and soaked with 2 ml of bacterial suspension; the "Control" groups were treated with 1–2 ml of sterile distilled water in similar manner. The filter papers of all Petri dishes were kept remain moist by adding a few drops of sterile distilled water whenever required.

It is already mentioned that the majority of species of the genus *Bacillus* will grow well on a rich medium such as nutrient agar (Collier *et al.*, 1998). The Bergey's Manual of Systematic Bacteriology written by Sneath *et al.* (1986) described that general morphology should be observed from young (24 hours) culture; a para sporal crystal (bodies) may be seen in the cells grown from 3–7 days (72 hours 168 hours) on nutrient agar. In the present studies a culture of 72 hours old and above was used in each series of experiments; which indicates that the 72 to 168 hours old culture of *B. thuringiensis* would mainly contain sporal and para-sporal bodies in or outside the vegetative cells.

The homogenous suspensions of about 29×10^8/ml and 25×10^8/ml viable rods/spores and crystals of 72 hrs and 168 hrs old irradiated surviving *B. thuringiensis* cultures were prepared and their pathogenicity was tested. The viable count obtained by pour-plate method (Cruickshank *et al.*, 1975) indicates only viability.

The workers of *H. indicola*, *M. championi* and nymphs of *B. beesoni* were divided separately into six groups. Each group had 25 termites. Group 1 of each species of termites was kept as control. While group 2 was infected by 72 hrs and 168 hrs old culture of non-irradiated *B. thuringiensis* separately. The groups 3, 4, 5 and 6 (of each species of termite) were infected by *B. thuringiensis* cultures exposed to 60 kr, 70 kr 100 kr and 150 kr doses of gamma rays, respectively. The experiments were replicated thrice.

Evaluation of experimental treatments was made by recording mortality of termites against gamma irradiated and non-irradiated *B. thuringiensis* at 24-hrs intervals till the 100% death of termites. Georghiou *et al.* (1975) used a formula for determining synergistic ratio of insecticides (synergistic ratio = LC_{50} insecticide alone ÷ LC_{50} insecticide in presence of synergist). In the present studies this formula was modified according to the nature of experiment and a term 'enhancement ratio' was used for keeping a uniformity among all the series of experiments.

$$\text{Enhancement ratio} = \frac{LT_{50} \text{ of non-irradiated } B.\ thuringiensis}{LT_{50} \text{ of irradiated } B.\ thuringiensis}$$

Treatment of data. The mortality percentage of termites, caused by *B. thuringiensis* infection was first corrected by the death among control groups, according to Abbotfs formula (1925) and its modification (Krejzova, 1975).

The formula was used when the mortality of control groups was from 5 to 16%; and if there were death of more than 16 % the experiments were discarded. The corrected data was plotted as regression lines. Adjustment in data was made by Probit analysis as mentioned by Goulden (1962) and Finney (1971). As in the present studies, there was zero to hundred percent response in all experiments thus maximum likelihood methods of Probit analysis was used as described by Fisher (1935) and Bliss (1938).

While considering analysis of the experiments, the result of such data were not usually included when the response of termites was between zero and ten or near 100 percent. The further details of the methods of Probit analysis has already been mentioned (Khan *et al.*, 1992).

Bacteriological diagnosis. Periodically dead termites were examined in nigrosin, spore stained and Gram stained smears. The presence of spores and crystal, particularly inside the vegetative cells was taken as presumptive evidence of *B. thuringiensis* action while its confirmation was also carried out very often by various chemical and fermentation tests as described by Breed *et al.* (1972) and MacFaddin (1976). In order to prove the pathogenicity of the irradiated or non-irradiated *B. thuringiensis* Koch's postulates were also followed as described by Bucher (1973). The virulence of *B. thuringiensis* was maintained by frequent pouring of its suspension into the Petri dishes containing the filter paper and nymphs of *B. beesoni* with subsequent re-isolation of the spore/crystal bearing bacteria.

Results

Bacillus thuringiensis very closely related to *B. anthracis* as well as to *B. cereus*, is a well known insect pathogen and, is also by far the most important in the biological control of insects (Sneath *et al.*, 1986). The characteristics *of Bacillus thuringiensis* show that it was glucose, starch and catalase positive, the hydrolysis of gelatin was occurred, utilization of citrate was noted and nitrate was reduced to nitrite. The bacteria were fairly motile with spores and crystalline bodies.

Survival of gamma irradiated *B. thuringiensis.* The viable count of 72 hrs, 96 hrs and 168 hrs old cultures of non-irradiated and gamma irradiated *B. thuringiensis* grown on nutrient agar medium is presented in Table I. The percentage survival and death (in whole number) of *B. thuringiensis* exposed to various doses of gamma rays is also tabulated (Table II).

Pathogenicity of 72 hrs old irradiated *B. thuringiensis.* A homogeneous suspension of about 29×10^8/ml viable rods/spores and crystals of *B. thuringiensis* was prepared from 72 hrs old cultures previously exposed to 60 kr, 70 kr, 100 kr and 150 kr doses of gamma rays. Non-irradiated 72 hrs old culture of *B. thuringiensis* was also used as control. The workers of *H. indicola*, *M. championi* and nymphs of *B. beesoni* were infected

Table I
Viable count of *B. thuringiensis* before and after irradiation at various doses of gamma rays

Age of Culture	Non irradiated *B. thuringiensis*	*B. thuringiensis* after irradiation with			
		60 kr	70 kr	100 kr	150 kr
72 hrs	290×10^7	240×10^7	211×10^7	180×10^7	130×10^7
96 hrs	300×10^7	231×10^7	210×10^7	175×10^7	120×10^7
168 hrs	250×10^7	210×10^7	195×10^7	170×10^7	135×10^7

Table II
Survival and death percentage of *B. thuringiensis* exposed to different doses of gamma rays

Radiation dose	72 hrs *B. thuringiensis* Survival/Death	96 hrs *B. thuringiensis* Survival/Death	168 hrs *B. thuringiensis* Survival/Death
60	83/17	77/20	84/16
70	73/27	70/30	78/22
100	60/40	58/42	68/32
150	45/55	40/60	54/46

Table III
Mortality percentage of *H. indicola* infected by 72 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	6.67	5.33	8.00	8.00	8.00
48	1.33	20.00	18.67	30.67	20.00	18.67
72	1.33	33.33	25.33	48.00	29.33	30.67
96	1.33	46.67	58.67	65.33	48.00	46.67
120	2.67	58.67	73.33	74.67	61.33	56.00
144	4.00	68.00	82.67	82.67	66.67	62.67
168	4.00	76.00	92.00	92.00	76.00	76.00
192	5.33	91.53	100.00	100.00	83.30	87.50
216	6.67	100.00			93.10	94.43
240	6.67				100.00	100.00

Table IV
Mortality percentage of *M. championi* infected by 72 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	8.00	9.33	9.33	5.33	5.33
48	0.00	20.00	17.33	16.00	9.33	12.00
72	0.00	33.33	28.00	32.00	16.00	29.33
96	0.00	42.67	37.33	44.00	24.00	33.33
120	0.00	53.33	53.33	54.67	32.00	40.00
144	0.00	61.33	65.33	68.00	42.37	49.33
168	0.00	72.00	73.33	70.67	53.33	56.00
192	0.00	80.00	85.33	81.33	61.33	65.33
216	0.00	89.33	92.00	88.00	69.33	77.33
240	0.00	100.00	100.00	92.00	77.33	88.00
264	1.33			100.00	90.67	100.00
288	1.33				97.33	
312	1.33				100.00	

by these irradiated and non-irradiated *B. thuringiensis* cultures and their mortality percentage are shown in Table III, IV and V, respectivcly. The LT_{50}, LT_{90} of these termites calculated by probit analysis is given in Table VI. The value of slopes of regression lines and their fiducial limits are also presented in the same table. The enhancement ratio of gamma irradiated *B. thuringiensis* tested against various species of termites, is shown in Table XI.

Pathogenicity of 168 hrs old irradiated *B. thuringiensis.* A homogenous suspension of about 25×10^8/ml viable rods/spores and crystals of 168 hrs old *B. thuringiensis* was prepared from each of the cultures already being exposed to different doses of gamma rays. Non-irradiated 168 hrs old culture of *B. thuringiensis* was used as control. The pathogenicity of the non-irradiated and irradiated culture of *B. thuringiensis* was tested against the three species of termites. The corrected percentage mortality of the workers of *H. indicola, M. championi* and nymphs of *B. beesoni* by 168 hrs old non-irradiated/irradiated cultures of *B. thuringiensis* are shown in Table VII, VIII and IX respectively. The LT_{50}, LT_{90} and value of slopes of regression lines, calculated by probit analysis are presented in Table X. While the enhancement ratio of gamma irradiated (168 hrs old) *B. thuringiensis* is shown in Table XI.

Table V
Mortality percentage of *B. beesoni* nymphs infected by 72 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	6.67	5.33	8.00	6.67	5.33
48	2.67	20.00	16.00	18.67	9.33	12.00
72	2.67	32.00	38.67	33.33	26.67	20.00
96	2.67	48.00	54.67	53.33	36.00	28.00
120	4.00	53.33	65.33	65.33	44.00	32.00
144	4.00	61.33	72.00	77.33	52.00	45.33
168	4.00	72.00	84.00	81.33	61.33	53.33
192	5.33	82.67	92.00	96.00	65.33	61.33
216	5.33	91.77	100.00	100.00	71.19	67.07
240	5.33	100.00			79.42	72.64
264	5.33				89.10	84.98
288	5.33				97.33	94.67
312	5.33				100.00	100.00

Table VI
LT_{50}/LT_{90} and slopes of regression lines of *H. indicola*, *M. championi* and *B. beesoni* infected by 72 hrs non-irradiated and irradiated *B. thuringiensis*

Doses of gamma rays	*Heterotermes*		*Microcerotermes*		*Bifiditermes*	
	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)
0 kr	91.1/225.0	3.3 ± 0.8	97.4/265.3	2.9 ± 0.7	96.3/249.4	3.1 ± 0.7
60 kr	82.3/169.5	4.1 ± 1.1	96.9/250.2	3.1 ± 1.4	86.3/195.8	3.6 ± 0.9
70 kr	69.7/165.7	3.4 ± 0.8	95.9/255.6	3.0 ± 0.7	82.8/197.5	3.4 ± 0.9
100 kr	92.0/233.9	3.2 ± 0.8	138.9/327.7	3.4 ± 1.2	121.4/319.2	3.0 ± 0.6
150 kr	92.8/234.0	3.2 ± 0.8	122.5/331.7	3.0 ± 0.7	137.3/358.9	3.1 ± 0.7

Table VII
Mortality percentage of *H. indicola* infected by 168 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	2.67	5.33	5.33	2.67	8.00
48	9.00	13.33	12.00	12.00	12.00	20.00
72	0.00	16.00	20.00	16.00	42.00	28.00
96	0.00	24.00	30.67	28.00	32.00	37.33
120	0.00	38.67	42.67	38.67	36.00	49.33
144	0.00	52.00	53.33	45.33	45.33	56.00
168	0.00	68.00	60.00	57.33	53.33	64.00
192	0.00	72.00	72.00	69.33	64.00	78.67
216	2.67	80.00	84.00	74.67	74.67	90.67
240	4.00	86.67	92.00	90.67	85.33	96.00
264	4.00	94.67	100.00	100.00	100.00	100.00
288	4.00	100.00				

Table VIII
Mortality percentage of *M. championi* infected by 168 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	4.00	4.00	8.00	4.00	9.33
48	0.00	16.00	12.00	13.33	8.00	20.00
72	0.00	26.67	20.00	22.67	12.00	36.00
96	1.33	38.67	29.33	33.33	20.00	41.33
120	1.33	45.33	37.33	41.33	28.00	60.00
144	1.33	54.67	45.33	49.33	36.00	69.33
168	1.33	61.33	58.67	61.33	44.00	82.67
192	1.33	70.67	77.33	72.00	53.33	92.00
216	1.33	84.00	81.33	84.00	65.33	97.33
240	1.33	89.33	89.33	92.00	73.33	100.00
264	2.67	93.33	94.67	100.00	82.32	
288	4.00	100.00	100.00		87.77	
312	4.00				96.00	
336	5.33				100.00	

Table IX
Mortality percentage of *B. beesoni* infected by 72 hrs non-irradiated and irradiated *B. thuringiensis*

Hrs after infection	Gr. 1 control	Gr. 2 non-irradiated *B. thuringiensis*	Gr. 3	Gr. 4	Gr. 5	Gr. 6
			B. thuringiensis after irradiation with			
			60 kr	70 kr	100 kr	150 kr
24	0.00	5.33	5.33	1.33	6.67	5.33
48	0.00	16.00	13.33	9.33	12.00	20.00
72	0.00	28.00	32.00	16.00	26.67	32.00
96	0.00	44.00	42.67	24.00	36.00	54.67
120	0.00	49.33	53.33	29.33	45.33	60.00
144	2.67	57.33	57.33	37.33	53.33	73.33
168	2.67	65.33	64.00	48.00	61.33	80.00
192	4.00	72.00	74.67	56.00	65.33	92.00
216	4.00	88.00	80.00	64.00	72.00	100.00
240	4.00	96.00	92.00	70.67	80.00	
264	4.00	100.00	100.00	82.67	88.00	
288	4.00			89.33	96.00	
312	4.00			100.00	100.00	

Table X
LT_{50}/LT_{90} and slopes of regression lines of *H. indicola*, *M. championi* and *B. beesoni* infected by 168 hrs non-irradiated and irradiated *B. thuringiensis*

Doses of gamma rays	*Heterotermes*		*Microcerotermes*		*Bifiditermes*	
	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)
0 kr	125.2/275.7	3.7 ± 0.8	113.5/282.4	3.2 ± 0.7	106.1/266.0	3.2 ± 0.8
60 kr	119.5/286.1	3.4 ± 0.8	123.8/281.1	3.6 ± 0.8	107.4/280.6	3.1 ± 0.7
70 kr	128.9/317.0	3.3 ± 1.3	116.8/303.0	3.1 ± 0.6	153.1/363.2	3.4 ± 0.8
100 kr	131.4/334.9	3.1 ± 0.8	154.6/353.4	3.6 ± 1.3	119.0/322.9	2.9 ± 0.7
150 kr	103.5/267.5	3.1 ± 0.7	87.0/210.9	3.3 ± 0.8	88.3/205.6	3.5 ± 0.8

Table XI
Enhancement ratio of gamma irradiated *B. thuringiensis* tested against *H. indicola*, *M. championi* and *B. beesoni*

Does of *B. thuringiensis*	*Heterotermes*		*Microcerotermes*		*Bifiditerms*	
	72 hrs	168 hrs	72 hrs	168 hrs	72 hrs	168 hrs
60 kr	1.107	1.048	1.005	0.917	1.116	0.988
70 kr	1.307	0.971	1.015	0.972	1.163	0.693
100 kr	0.990	0.952	0.710	0.734	0.793	0.891
150 kr	0.982	1.209	0.795	1.320	0.701	1.201

Discussion

Bacillus thuringiensis is distinguished from *B. cereus* by the production of a crystalline protein body in the cell during the phase of spore formation (Gibson and Gordon, 1974). In the present studies, it was also noted that *B. thuringiensis* rods usually contain spores and crystal in the crushed smear of freshly dead infected termites. However, the spores/crystals of *B. thuringiensis* were seen mostly librated outside the cells in 72 hrs and 168 hrs old cultures. Aizawa (1971) already discussed that problems of pathogenicity, virulence and strain selection are important in applied insect microbiology.

The results of the present studies showed that all the cultures of *B. thuringiensis* survived following exposure from 60 to 150 kr of gamma rays. A much higher dose of gamma rays may reduce the virulence of *B. thuringiensis* as reported by Becker (2002) that radiation at a dose of 20–25 kGy caused a 20% to 30% reduction in the effectiveness of Bti powder against mosquito larvae. An analysis of results showed that the survival of *B. thuringiensis* was dose dependent. The survival of 72 hrs, 96 hrs and 168 hrs old cultures of *B. thuringiensis* decreased with an increase in dose of gamma rays. It was also noted that 72 hrs and 96 hrs irradiated *B. thuringiensis* cultures were more sensitive to the given doses of gamma rays than 168 hrs irradiated ones.

The data of LT_{50} and LT_{90} pertaining to the pathogenicity of 72 hrs old culture of non-irradiated and irradiated *B. thuringiensis* against *H. indicola*, *M. championi* and *B. beesoni* showed that 60 kr and 70 kr irradiated bacteria caused quicker mortality rate than the non-irradiated one. In all these cases enhancement ratio was more than 1.048. While 100 kr and 150 kr irradiated *B. thuringiensis* caused slower mortality rate than the non-irradiated one. These results indicate that 60 kr and 70kr irradiated *B. thuringiensis* were more pathogenic than the 100 kr and 150 kr irradiated and non-irradiated ones.

The pathogenicity of 168 hrs old irradiated *B. thuringiensis* was tested at the time when bacterial cultures mainly consisted of spores/crystals only. The data showed that in case of *H. indicola*, *M. championi* and *B. beesoni,* 150 kr irradiated *B. thuringiensis* caused quicker mortality rate than the non-irradiated one. While 60 kr 70 kr and 100 kr irradiated *B. thuringiensis* caused slower mortality rate than the non-irradiated one. This observation indicated that 150 kr, 168 hrs old irradiated *B. thuringiensis* was more pathogenic than 60 kr, 70 kr and 100 kr, 168 hrs old irradiated and non-irradiated bacterial cultures. Studies of Merdan *et al.* (1975) showed that gamma radiation had no significant effect on the bacterial virulence on the Eri-silk worm, *Philosamia ricini* larvae at the level of 80 krads. The virulence, however, increased significantly when irradiated with gamma rays at the level of 100 krads. If *Bacillus* strains are grown under conditions where spore formation occurs, their long term maintenance does not cause any problems. Sporulated cultures, preferable on agar slants and protected from drying, can be kept at room temperature or below, for at least 1 year. Many strains will survive for 10 years or more under these conditions (Sneath *et al.*, 1986).

The present studies also showed that virulence of *B. thuringiensis* depended on the age of the culture exposed to given doses of gamma rays and tested against various species of termite. Comparative analysis of the data showed that *H. indicola* workers were most susceptible to 72 hrs old culture of irradiated *B. thuringiensis* as compared to the workers of *M. championi* and nymphs of *B. beesoni.* On the other hand, nymphs of *B. beesoni* were most susceptible to 168 hrs old irradiated *B. thuringiensis* as compared to the workers of *H. indicola* and *M. championi* (except in 70 kr). In general, it appeared that each species of termite had its own susceptible response to *B. thuringiensis* culture exposed to different doses of gamma rays.

Acknowledgement. This work was supported by P.L.- 480 Grant F.G. Pa-201 (Project A 17. FS 21).

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