▼ Journal of Research (Science), Bahauddin Zakariya University, Multan, Pakistan.
Vol.17, No.1, January 2006, pp. 35-43 ISSN 1021-1012

ENHANCEMENT OF VIRULENCE OF *BACILLUS THURINGIENSIS* AND *SERRATIA MARCESCENS* BY CHEMICALS

Khalid Idrees Khan
University College of Pharmacy, University of the Punjab, Lahore, Pakistan.

Abstract: Studies were conducted on the enhancement of pathogenicity of *Bacillus thuringiensis* by 1% boric acid against various species of termites. The increase in virulence of *Serratia marcescens* by 1% Potassium chloride or 1% Sodium citrate against the workers of *M. championi* has also been established. The increase in virulence is confirmed by the enhancement ratios, which are ranging from about 1.5 to 1.8 for *Bacillus thuringiensis* and 1.3 to 1.6 for *Serratia marcescens*. It was also noted that 1% boric acid alone was found toxic to various species of termites. However, Potassium chloride and Sodium citrate in a concentration of 1% were non-toxic to the workers of *M. championi*.

Keywords: *Bacillus thuringiensis*, Chemicals, *Serratia marcescens*, virulence.

INTRODUCTION

Boric acid is toxic to certain insects. It is efficient to control house-flies in manure [Midgley and Dunklee 1943]. Bare [1945] has also reported that boric acid is used as effective poison bait for the German cockroach. Doane and Wallis [1964] tested a number of chemical compounds to see the effects on the pathogenicity of *Bacillus thuringiensis*. A combination of boric acid and *B. thuringiensis* resulted in an increased mortality of gypsy moth, *Prothetria dispar* larvae. In preliminary testing, Steinhaus [1959] obtained evidence that the combination of acetic acid and *Serratia marcescens* might increase the mortality rate of grater wax moth *Galleria mellonella*.

It has been reported that *Serratia marcescens* and *Bacillus thuringiensis* was potentially pathogenic to various species of termites [Khan *et al.* 1977, Khan 1981 and Khan *et al.* 1985]. Thus, the present studies were carried out in order to explore the possibility of enhancing the pathogenicity of *Bacillus thuringiensis* and *Serratia marcescens* by different chemicals.

MATERIALS AND METHODS

A series of tests were designed to determine the pathogenicity of *Bacillus thuringiensis* and *Serratia marcescens* cultures used with or without a given chemical. The tests were carried out as described by Doane and Wallis [1964].

PATHOGENICITY OF *BACILLUS THURINGIENSIS* WITH OR WITHOUT 1% BORIC ACID

Bacillus thuringiensis serotype 3a, 3b was grown on nutrient agar medium for 72 hrs. A homogeneous suspension of about $15x10^7$ ml^{-1} viable rod

was prepared in sterile distilled water. An amount of 100mg of boric acid was added in 10ml of the above bacterial suspension. In this way the concentration of boric acid in the suspension of *B. thuringiensis* was one percent. A solution of boric acid in a concentration of 1% was also prepared in sterile distilled water. The pathogenicity of these suspensions was tested against various species of termites.

The workers of *O. obesus, M. championi* and nymphs of *B. beesoni* were divided into four groups. Each group had 25 termites. Group 1 of each species of termite was kept as control. The group 2 each of the above mentioned termite was infected by one ml of *B. thuringiensis* suspension prepared in sterile distilled water. However, group 3 of each species of termites was fed on a filter paper soaked in 1% boric acid solution. On the other hand group 4 of *O. obesus, M. championi* and *B. beesoni* was infected by one ml of bacterial suspension mixed with 1% boric acid. The experiments were replicated thrice.

PATHOGENICITY OF *S. MARCESCENS* WITH OR WITHOUT 1% POTASSIUM CHLORIDE AND SODIUM CITRATE

Serratia marcescens was grown on nutrient agar medium at 32^{o}C for 72 hours. A homogeneous suspension of about 15x10^{7} ml^{-1} viable rods was prepared in sterile distilled water. One ml of this suspension was added to one ml sterile solution of 2% potassium chloride. Similarly, one ml of the bacterial suspension was added to one ml of sterile solution of 2% sodium citrate. In this way the concentration of potassium chloride and sodium citrate was one percent in each of the above mentioned solutions. However, one ml of *S. marcescens* suspension was added to one ml of sterile water only. The pathogenicity of these suspensions was tested against *M. championi*.

Microcerotermes championiu workers were divided into six groups. Each group had 25 termites. Group 1 was kept as control. The termites of group 2 were fed on a filter paper soaked in 1% potassium chloride. The workers of *M. championi* of group 3 were fed in a filter paper soaked in 1% sodium citrate solution only. The group 4 was infected with one ml of *S. marcescens* suspension alone. The group 5 was infected with one ml of bacterial suspension containing 1% potassium chloride. The group 6 was infected with one ml of bacterial suspension containing 1% sodium citrate. The experiments were replicated thrice.

RESULTS

ENHANCEMENT OF PATHOGENICITY OF *B. THURINGIENSIS* BY BORIC ACID

A homogenous suspension of about 15x10^{7} ml^{-1} viable rods of 72 hrs old *B. thuringiensis* culture was prepared. It was given to each species of termites. Another group of termites was given 1% boric acid. The third group of termites was given a mixture of *B. thuringiensis* and 1% boric

acid. *Odontotermes obesus, M. championi* and *B. beesoni* were used as test insects. Preliminary experiments were also carried out where 1% boric acid was used as an additive and in substrate. The results of these experiments showed that boric acid used as an additive, was more effective in enhancing the action of *B. thuringiensis* than when used in substrate. Thus in the present study experiments were designed to see the effect of 1% boric acid used as an additive only.

In case of *O. obesus*, there was about 19%, 29% and 49% mortality of termites at 24 hrs following infection by *B. thuringiensis*, 1% boric acid and a mixture of both, respectively. There was 100% death of *O. obesus* at 120 hrs, 96 hrs and 72 hrs following infection by *B. thuringiensis*, 1% boric acid and mixture of both, respectively (Table 1).

The LT_{50}, LT_{90}, value of slopes of regression lines, calculated by probit analysis and enhancement ratio are presented in Table 4. The regression lines and their equations are shown in Fig. 1. *Odontotermes obesus* workers, infected by *B. thuringiensis*, 1% boric acid and a mixture of both showed LT_{50} at 45 hrs, 34 hrs and 25 hrs and LT_{90} at 101 hrs, 69 hrs and 56 hrs, respectively (Table 4).

Table 1: Mortality percentage of workers of *O. obesus* treated with dilutions of *B. thuringiensis* with and without Boric acid

Hours after infection /treatment	Group 1 Control	Group 2 *B. thuringiensis*	Group 3 Boric acid	Group 4 *B. thuringiensis* + Boric acid
24	02.67	18.67	29.33	49.33
48	14.67	53.17	67.17	79.72
72	16.00	73.01	90.47	100.00
96	16.00	85.71	100.00	
120	16.00	100.00		

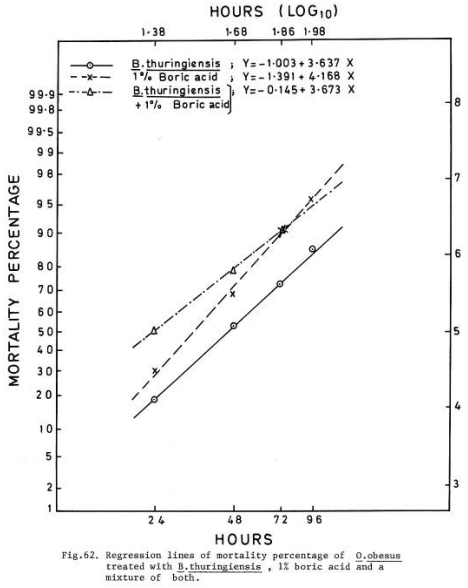

Fig. 1: Regression lines of mortality percentage of *O. obesus* treated with *B. thuringiensis*, 1% boric acid and a mixture of both.

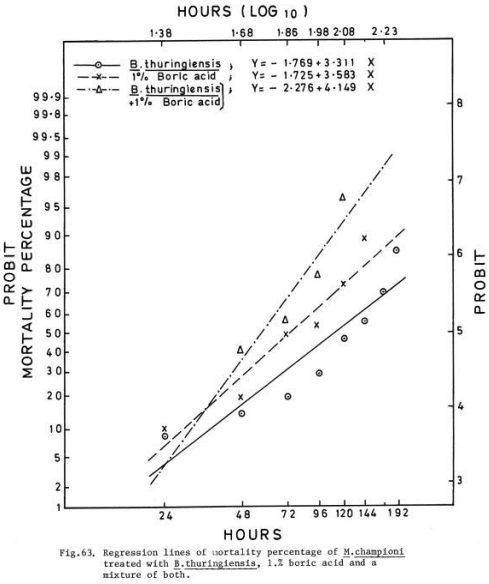

Fig. 2: Regression lines of mortality percentage of *M. championi* treated with *B. thuringiensis*, 1% boric acid and a mixture of both.

In case of *M. championi*, there was about 19%, 47% and 56% mortality at 72 hrs following infection by *B. thuringiensis*, 1% boric acid and mixture of both, respectively (Table 2). There was 100% death of *M. championi* at 216 hrs, 168 hrs and 144 hrs following infection by *B. thuringiensis*, 1% boric acid and a mixture of both, respectively (Table 2). The LT_{50}, LT_{90} value of slopes of regression lines and enhancement ratio are presented in Table 4. While the regression lines and their equations are shown in Fig. 2. *Microcerotermes championi* workers, infected by *B. thuringiensis*, 1% boric acid and a mixture of both showed LT_{50} at about 111 hrs, 75 hrs and 57 hrs, LT_{90} occurred at 270 hrs, 171 hrs and 115 hrs, respectively.

Table 2: Mortality percentage of workers of *M. championi* treated with dilutions of *B. thuringiensis* with and without Boric acid

Hours after infection /treatment	Group 1 Control	Group 2 *B. thuringiensis*	Group 3 Boric acid	Group 4 *B. thuringiensis* + Boric acid
24	0.00	8.00	9.33	8.00
48	0.00	13.33	18.67	40.00
72	0.00	18.67	46.67	56.00
96	0.00	28.00	52.00	76.00
120	0.00	44.00	72.00	96.00
144	0.00	60.00	88.00	100.00
168	4.00	73.33	100.00	
192	4.00	85.33		
216	4.00	100.00		

Table 3: Mortality percentage of nymphs of *B. beesoni* treated with dilutions of *B. thuringiensis* with and without Boric acid

Hours after infection /treatment	Group 1 Control	Group 2 *B. thuringiensis*	Group 3 Boric acid	Group 4 *B. thuringiensis* + Boric acid
24	0.00	4.00	8.00	6.67
48	1.33	9.33	14.67	30.67
72	2.67	12.00	40.00	41.33
96	4.00	36.00	62.67	60.00
120	4.00	60.00	68.00	76.00
144	4.00	68.00	77.33	88.00
168	4.00	72.00	85.33	97.33
192	4.00	84.00	92.00	100.00
216	4.00	93.33	100.00	
240	4.00	100.00		

Table 4: LT_{50} / LT_{90} and slopes of Regression lines of *O. obesus*, *M. championi* and *B. beesoni* caused *B. thuringiensis*, 1% Boric acid and a mixture of both.

Infection given by	Odontotermes		Microcerotermes		Bifiditermes	
	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)	LT_{50}/LT_{90}	Slope (b)
B. thuringiensis	44.7/100.6	3.6±1.1	110.7/269.6	3.3±0.8	108.0/221.8	4.1±2.0
1% Boric acid	34.2/69.3	4.2±1.4	75.3/171.4	3.6±0.9	80.6/188.2	3.5±0.9
B. thuringiensis + 1% Boric acid	25.2/56.1	3.7±1.6	56.7/115.4	4.1±1.3	71.5/156.2	3.8±0.9
Enhancement Ratio*	1.774		1.952		1.510	

* Enhancement Ratio = $\frac{LT_{50} \text{ of } B. \textit{ thuringiensis} \text{ alone}}{LT_{50} \text{ of the mixture of } B. \textit{ thuringiensis} \text{ and boric acid}}$

In case of *B. beesoni*, there was 12%, 40% and 41% mortality at 72 hrs following *B. thuringiensis*, 1% boric acid, and a mixture of both,

respectively. There was 100% death of *B. beesoni* at 240 hrs, 216 hrs and 192 hrs following infection of *B. thuringiensis*, 1% boric acid and a mixture of both, respectively (Table 3).

The LT_{50}, LT_{90}, regression slopes and enhancement ratio are presented in Table 4. While the regression lines and their equations are shown in Fig. 3. *Bifiditermes beesoni* nymphs infected by *B. thuringiensis*, 1% boric acid and a mixture of both showed LT_{50} at about 108 hrs, 81 hrs and 71 hrs and LT_{90} was calculated as about 222 hrs, 188 hrs and 156 hrs, respectively.

It was noted that mixture of *B. thuringiensis* and boric acid was more effective in causing the death of termites than the each one when used alone. Various species of termites showed different response to *B. thuringiensis* used alone or in mixture with 1% boric acid.

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 uniformity among all the series of experiments.

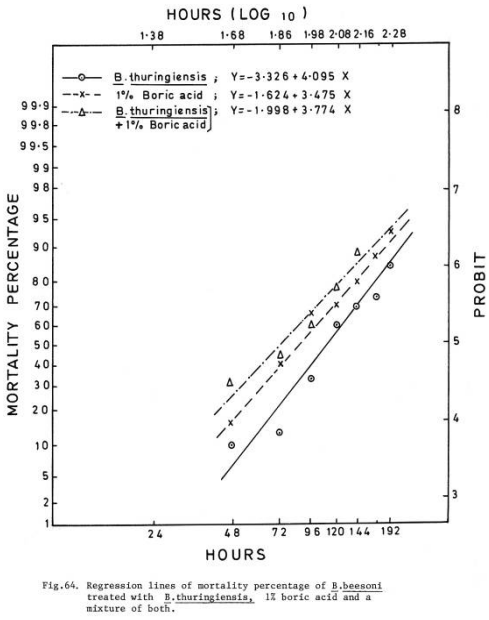

Fig.64. Regression lines of mortality percentage of B.beesoni treated with B.thuringiensis, 1% boric acid and a mixture of both.

Fig. 3: Regression lines of mortality percentage of *B. beesoni* treated with *B. thuringiensis*, 1% boric acid and a mixture of both.

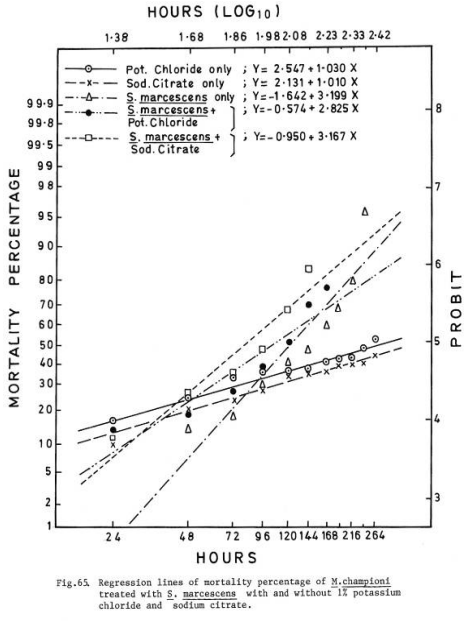

Fig.65. Regression lines of mortality percentage of M.championi treated with S. marcescens with and without 1% potassium chloride and sodium citrate.

Fig. 4: Regression lines of mortality percentage of *M. championi* treated with *S. marcescens* with or without 1% KCl and sodium citrate.

ENHANCEMENT OF PATHOGENICITY OF *S. MARCESCENS* BY POTASSIUM CHLORIDE AND SODIUM CITRATE

A homogenous suspension of about $15x10^7$ ml^{-1} viable of 72 hrs old culture of *S. marcescens* was prepared in sterile distilled water. Workers of *M. championi* were infected by *S. marcescens* (alone), *S. marcescens*

in solution of 1% potassium chloride and *S. marcescens* in a solution of 1% sodium citrate. Solutions of potassium chloride and sodium citrate (1% each) were also used against *M. championi* as control. In preliminary experiments, potassium chloride and sodium citrate were used as an additive and in substrate against workers of *M. championi.* The results of these experiments showed that the above mentioned chemicals, used as an additive were more effective in enhancing the pathogenicity of S. *marcescens* than when used in substrate. Thus in the present study, experiments were designed to see the effects of 1% potassium chloride and sodium citrate used as an additive.

There was about 33% and 24% death of *M. championi* at 72 hrs following treatment of 1% potassium chloride and 1% sodium citrate, respectively (Table 5). While at 264 hrs there was about 55% and 45% death of termites in the above respective group. The termites of these two groups survived for a month, they were then discarded. However, there was about 19%, 27% and 36% mortality of *M. championi* at 72 hrs following infection by *S. marcescens* used alone or with a mixture of potassium chloride and with a mixture of sodium citrate, respectively. There was 100% death of *M. championi* at 264 hrs, 192 hrs and 168 hrs following infection of *S. marcescens* alone, *S*. *marcescens* plus potassium chloride and *S. marcescens* plus sodium citrate, respectively (Table 5).

Table 5: Mortality percentage of workers of *M. championi* treated with dilutions of *S. marcescens* with and without 1% Potassium chloride and Sodium citrate

Hours after Gr 1 infection control	Group 1 Control	Group 2 Potassium chloride	Group 3 Sodium citrate	Group 4 *S. marcescens*	Group 5 *S. marcescens* + Pot. chloride	Group 6 *S. marcescens* + Sod. citrate
24	0.00	16.00	10.67	6.67	13.33	12.00
48	0.00	25.33	20.00	14.67	18.67	28.00
72	0.00	33.33	24.00	18.67	26.67	36.00
96	0.00	37.33	26.67	30.67	40.00	48.00
120	0.00	37.33	37.33	40.00	52.00	72.00
144	0.00	37.33	37.33	48.00	72.00	85.33
168	0.00	42.67	37.33	61.33	77.33	100.00
192	2.67	46.67	40.00	72.00	100.00	
216	2.67	46.67	40.00	80.00		
240	2.67	49.33	41.33	96.00		
264	2.67	54.67	45.33	100.00		

The LT_{50}, LT_{90} and value of slopes of regression lines calculated by statistical analysis and enhancement ratio are presented in Table 4. While regression lines and their equations are shown in Fig. 4. *Microcerotermes championi* workers infected by *S. marcescens* alone or with 1% potassium chloride and with 1% sodium citrate showed LT_{50} at about 119 hrs, 94 hrs and 76 hrs; and LT_{90} at about 300 hrs, 267 hrs and 192 hrs, respectively. On the other hand there was 50% death of *M. championi* at 241 hrs and 342 hrs by 1% potassium chloride and sodium citrate each used alone, respectively. However, 90% death of termites was expected to occur after about six months as calculated by probit analysis (Table 6).

The above mentioned results showed that potassium chloride and sodium citrate were not toxic to the workers of *M. championi* (as the value of slope is also only one). However, when they are mixed with *S. marcescens* they caused an increase in the virulence of the bacteria.

Table 6: LT_{50}/LT_{90} and slopes of regression lines of *M. championi* treated with *S. marcescens* with and without 1% Potassium chloride and Sodium citrate.

Treatment given by	LT_{50} / LT_{90}	Slope (b)	Enhancement ratio
Potassium chloride (control)	240.7/4207.3	1.0±0.5	-
Sodium citrate (control)	343.3/6354.3	1.0±0.6	-
Serratia marcescens	119.2/299.5	3.2±1.5	-
S. marcescens +Potassium chloride	94.0/266.7	2.8±0.7	1.268
S. marcescens + Sodium citrate	75.6/191.8	3.2±0.8	1.577

DISCUSSION

Doane and Wallis [1964] treated larvae of the gypsy moth, *porthetria dispar* (Linnaeus), with combination of 1% boric acid and *Bacullus thuringiensis* var. *thuringiensis* Berliner and with each used alone. The addition of boric acid was consistently followed by a marked increase in larvae mortality as compared with that when *B. thuringiensis* was used alone. The mortality of larvae feeding on leaves treated with only 1% boric acid was about equal to these in untreated controls. The reason for the increased effectiveness of the combination has not been determined.

Boric acid used in sufficient quantity is toxic to certain insects. It is an efficient house-fly larvacide used at the rate of 5 lb/ton of manure [Midgley and Dunklee 1943]. Bare [1945] found 10% boric acid in confectioner's sugar and effective poison bait for the German cockroach.

Keeping in view the above mentioned studies, 1% boric acid was used with *B. thuringiensis*. At first in preliminary experiments (not mentioned here) when 1% boric acid was added in substrate, there was normal growth of bacteria on the medium. It means that 1% boric acid was not toxic to *B. thuringiensis*. Moreover, this bacterial culture did not show an increase in pathogenicity to various species of termites. Further studies showed that 1% boric acid caused mortality of termites earlier than *B. thuringiensis*. However, when 1% boric acid was mixed with 72 hrs old culture of *B. thuringiensis* there was still earlier mortality of termites, as indicated by LT_{50}, LT_{90} and enhancement ratio (Table 4). It could not be ascertained whether boric acid acted directly on the spore, on the crystal or in some undetermined manner to the gut wall of termites. Doane and Wallis [1964] were also unable to present any explanation for their studies on enhancement of the action of *B. thuringiensis* var. *thuringiensis* Berliner by 1% boric acid. Anyhow, the present studies suggest that 1% boric acid alone can also be used to control the termites effectively.

On the other hand, Stainhaus [1959] reported that when *Serratia marcescens* is suspended in a solution of dilute acetic and microfed to *Gallaria* larvae, the resulting mortality from infection may at times shows some increase over that resulting from the administration of *S*. *marcescens* alone. Doan and Wallis [1964] listed a number of compounds including organic and inorganic acids, against the larvae of the gypsy moth for general effects and for their influence on the pathogenicity of *B*. *thuringiensis.* Ascorbic acid, sodium citrate and potassium chloride were tested at 1% concentration.

In accordance with the above mentioned studies, preliminary experiments were conducted to see the action of potassium chloride and sodium citrate on the growth of *S*. *marcescens* in vitro. It was noted that when these chemicals were incorporated separately into the medium (substrate), they were found nontoxic to *S*. *marcescens*. In further studies it was noted that 1% potassium chloride or 1% sodium citrate, when each was used alone, caused mortality of *M*. *championi* over a longer period as compared to *S. marcescens*. While a mixture of *S. marcescens* and 1% potassium chloride or 1% sodium citrate, caused mortality of termites in a shorter period of time as compared to *S. marcescens* alone. These observations suggested that the use of either 1% potassium chloride or sodium citrate with *S. marcescens* cultures enhanced the action of *S*. *marcescens* in causing the mortality of termites. The data of LT_{50} LT_{90} and enhancement ratio (Table 6) showed that 1% sodium citrate when mixed with *S. marcescens* caused quicker rate of mortality of termite as compared to the mixture of 1% potassium chloride and *S. marcescens*.

Benz [1971] presented classification of synergism. According to him, there is supplemental synergism. He described it a system of two effective components which together produce an effect greater than algebraic sum of the single effect. In the present studies it was seen that a combination of *B. thuringiensis* and boric acid was more effective in causing the death of the termites. The joint action of 1% boric acid and *B*. *thuringiensis* may be regarded as supplemental synergism.

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