Additive Effect of Certain Chemicals on the Efficacy of *Bacillus thuringiensis* var. *kurstaki* Ber. Against *Spodoptera litura* (Fab.)

E. Sree Latha, P.V. Krishnayya and G.V. Subbaratnam

Department of Entomology, Agricultural College, Bapatla 522 101, India

ABSTRACT

Four groups of chemical *viz.*, inorganic salts (calcium carbonate and calcium sulphate), protein solubilizing agents (tween 80 and sodium dodecyl sulphate), amino acids (aspartic acid and lysine) and amides (acetamide and benzamide) were tested @ 0.05% for their additive effect on activity of *Bacillus thuringiensis* var. *kurstaki* (*Btk* dipel 8L) against *Spodoptera litura*. The median lethal concentrations were fixed after allowing third instar larvae of *Spodoptera. litura* to feed on treated castor leaves continuously for three days in the laboratory. Addition of these chemicals increased the efficacy of *Btk* by reducing the LC_{50} values by 1.80 to 2.83 fold. Additive chemicals potentiated the insecticidal activity of *Btk* by resulting in quick toxic effect, increased inhibition of feeding, reduction in larval and pupal weights, malformation of pupae and adults and reduction in adult emergence.

Key words : Additive Chemicals, *Bacillus thuringiensis*, Potentiation, *Spodoptera litura*

The rapidly increasing magnitude and complexity of insecticidal resistance problems and hazards involved in their use are forcing investigation on alternate control measures and increasing interest is being taken in the possible role of insect pathogens in pest management in recent years. Among the insect pathogens studied so far, *Bacillus thuringiensis* var. *kurstaki* Ber (*Btk)* has been extensively investigated and was found effective against *Spodoptera litura* (Fab.). However peritrophic membrane of the mid gut and low pH of gut juice are responsible for less susceptibility of *S. litura* to *Bt* (Narayanan *et al.*, 1976). At this juncture, biochemical means to enhance the activity of *Bt* by the addition of non toxic compounds were developed. Thereafter, studies were conducted on potentiating effect of certain additive chemicals like inorganic salts, protein solubilizing agents, amino acids and amides in combination with *Bt* against *Spodoptera littoralis* (Boisd), *Agrotis ipsilon* (Hub.) and *Mamestra configurata* (Walker) (Salama *et al.*, 1986; 1989 & Morris *et al.*, 1995). Keeping this in view, the present investigation on additive effect of certain chemicals on the efficacy of *Btk* against *S. litura* was taken up during 1996-97.

Materials and Methods

The investigations were carried out to evaluate the insecticidal activity of *Btk* (dipel 8L, 17,600 IU/mg) alone and in combination with additive chemicals @ 0.05%) belonging to four groups *viz.*, inorganic salts (calcium carbonate ($CaCO_3$) and calcium sulphate ($CaSO_4$)), protein solubilizing agents (tween 80 and sodium dodecyl sulphate (SDS)), amino acids (aspartic acid and lysine) and amides (acetamide and benzamide) against the larvae of *S. litura*. The median lethal concentrations (LC_{50}) of *Btk* (Strain H.D-1, 3a, 3b) alone and in combination with additive chemicals were calculated against third instar larvae of *S. litura* using leaf disc (50 cm^2) dip (10 seconds) method (Deshmukh & Mathai, 1991). The different concentrations of *B.t.k* and additive chemicals were prepared (vol./vol.) in distilled water. The larvae were fed with *Btk* treated castor leaf discs for three days and afterwards untreated leaves were given as food. Daily larval mortalities were

recorded and cumulative larval mortality upto pupation was used for calculation of LC_{50} values by probit analysis. (Finney, 1981). The estimated LC_{50} values of *Btk* alone and its combinations with additive chemicals were fed to the third instar larvae for three days to study their effect on larval mortality, feeding inhibition, weight loss of larvae and pupae, pupal and adult malformations and reduction in adult emergence. Larval mortality was recorded every day after the *Btk* treatment throughout the larval period and the corrected larval mortality were calculated and expressed as % cumulative larval mortality. *Btk* treated 50 cm^2 leaf discs were supplied to the larvae as per the requirement and the left over portion of the leaf area was calculated to estimate the % inhibition of feeding over control during the three days of *Btk* treatment. After the *Btk* treatment i.e. from fourth day onwards live larvae were weighed daily till pupation and the pupal weights were recorded one day after pupation to estimate % weight reduction of the larvae and pupae over control. % malformed pupae and adults, and reduction in adult emergence over control was also recorded in each treatment. The experiment were conducted with five replications with 10 larvae/replication in completely R.B.D. The data obtained were analysed by using ANOVA and to % larval mortality, malformed pupae and adults, adult emergence, inhibition of feeding and reduction in larval and pupal weights were subjected to arc sin percentage transformation and the variance was calculated.

Results and Discussion

LC_{50} Values : The LC_{50} values calculated were in the descending order of 0.1713, 0.0950, 0.0854, 0.0790, 0.0738, 0.0728, 0.0700, 0.0608 and 0.0606% vol/vol. resulted from the treatments of *Btk* alone and combination of *Btk* with the additive chemicals benzamide, $CaSO_4$, aspartic acid, acetamide, tween 80, lysine, SDS and $CaCO_3$, respectively. The decrease in LC_{50} values of *Btk* against *S. litura* larvae due to the addition of additive chemicals was between 1.80 and 2.83 times (Table 1). The potentiating effect observed was in agreement with the earlier observations of Salama *et al.* (1986 & 1989) and Morris *et al.* (1995) against *S. littoralis, A. ipsilon* and *M. configurata.*

Effect on Mortality of Larvae : The larval mortalities on the 1st day after completion of three days of *Btk* treatment were significantly high in *B.t.k* + $CaCO_3$ (8.0%) and *Btk* + SDS (7.4%). The same treatments were superior to other treatments in resulting in higher % larval mortality on 3rd day (36.2 and 42.2) also. However, on 6th day after the *Btk* treatment and combinations were on par with each other in resulting in higher larval mortalities over control (Table 1). Early and higher larval mortality in combination treatments compared to *Btk* alone suggests that the additive chemicals might have increased the insecticidal activity of *Btk* Further, higher larval mortalities in *Btk* + $CaCO_3$ and *Btk* + SDS were because of the individual toxic effects of $CaCO_3$ and SDS i.e. 14.4 and 9.4% larval mortality, respectively.

Effect on Larval Feeding Inhibition : The % inhibition of larval feeding during 1st day of the treatment was significantly high in *Btk* + $CaCO_3$ (28.7) and *Btk* + SDS (27.2). The same treatment were superior to other treatments in resulting in higher % inhibition of larval feeding on 2nd day (33.4 and 33.8) and 3rd day (45.6 and 49.6) respectively. Besides these two treatments, the % inhibition of larval feeding recorded on third day were in the descending order of *Btk* + $CaSO_4$ (39.6), *Btk* + lysine (38.0), *Btk* + tween 80 (37.2), *Btk* + acetamide (35.6), *Btk* + aspartic acid (34.8), *Btk* + benzamide (34.4) and *Btk* alone (32.4). In all the treatments the % inhibition of larval feeding increased gradually from Ist to 3rd day during the treatment (Table 1). The target site of activity of *Bt* protein was midgut, which is prime centre for food digestion and assimilation. Therefore, feeding inhibition was the immediate affect. The synergistic effect on larval feeding inhibition due to additive chemicals was due to their individual effect, particularly $CaCO_3$ and SDS recorded a maximum of 23.2 and 22.4 % reduction in food consumption over control.

Table 1. Effect of *Btk* with additive chemicals (0.05%) at their LC_{50} values on mortality and feeding inhibition of *Spodoptera litura* larvae

Treatments	% cumulative larval mortality over control-days after treatment			% inhibition of larval feeding over control-days during the treatment		
	1st	3rd	6th	1st	2nd	3rd
$CaCO_3$ 0.05%	5.2 (13.1)^{b}	13.2 (21.3)^{e}	14.4 (22.3)^{b}	12.3 (20.5)^{f}	19.6 (26.3)^{fg}	23.2 (28.8)^{f}
$CaSO_4$ 0.05%	2.2 (8.5)^{cd}	4.6 (12.3)^{f}	4.6 (12.4)^{d}	7.3 (15.8)^{h}	10.2 (18.6)^{h}	14.5 (22.4)^{g}
Tween 80 0.05%	3.0 (10.0)^{c}	5.8 (13.9)^{f}	5.8 (13.9)^{d}	6.6 (14.9)^{hi}	10.2 (18.6)^{h}	14.6 (22.5)^{g}
SDS 0.05%	4.6 (12.4)^{b}	7.0 (15.4)^{f}	9.4 (17.9)^{c}	10.1 (18.5)^{g}	17.0 (24.3)^{g}	22.4 (28.2)^{f}
Aspartic acid 0.05%	0.0 (4.05)^{e}	4.6 (12.3)^{f}	4.8 (12.7)^{d}	0.0 (4.05)^{k}	3.0 (9.9)^{j}	5.9 (14.0)^{i}
Lysine 0.05%	0.0 (4.05)^{e}	4.6 (12.3)^{f}	4.7 (12.5)^{d}	0.0 (4.05)^{k}	3.4 (10.4)^{j}	5.0 (13.0)^{i}
Acetamide 0.05%	1.8 (7.7)^{d}	5.4 (13.4)^{f}	5.4 (13.4)^{d}	4.6 (12.4)^{i}	6.6 (14.8)^{i}	9.1 (17.6)^{h}
Benzamide 0.05%	2.6 (9.3)^{e}	5.0 (12.9)^{f}	5.1 (13.1)^{d}	3.9 (11.4)^{i}	5.6 (13.7)^{i}	7.8 (16.3)^{h}
Btk 0.1713%	2.8 (9.5)^{e}	17.6 (24.8)^{e}	53.0 (46.6)^{a}	20.8 (27.2)^{cd}	24.8 (29.8)^{cde}	32.4 (34.7)^{e}
Btk 0.0606% + $CaCO_3$ 0.05%	8.0 (16.3)^{a}	36.2 (37.0)^{ab}	48.8 (44.5)^{a}	28.7 (32.4)^{a}	33.4 (35.3)^{a}	45.6 (42.5)^{b}
Btk 0.0854% + $CaSO_4$ 0.05%	4.4 (12.1)^{b}	26.8 (31.1)^{d}	48.9 (44.4)^{a}	23.8 (29.2)^{bc}	25.2 (30.1)^{cde}	39.6 (39.0)^{c}
Btk 0.0728% + Tween 80 0.05%	4.2 (11.9)^{b}	34.0 (35.7)^{b}	52.0 (46.2)^{a}	23.7 (29.1)^{bc}	28.0 (32.2)^{bc}	37.2 (37.6)^{cd}
Btk 0.0608% + SDS 0.05%	7.4 (15.9)^{a}	42.2 (40.5)^{a}	49.0 (44.5)^{a}	27.2 (31.4)^{ab}	33.8 (35.5)^{a}	49.6 (44.8)^{a}
Btk 0.0790% + Aspartic acid 0.05%	3.0 (10.0)^{c}	32.8 (34.9)^{bc}	52.4 (46.3)^{a}	18.1 (25.2)^{de}	22.4 (28.2)^{ef}	34.8 (36.1)^{de}
Btk 0.0700% + Lysine 0.05%	3.0 (10.0)^{c}	34.2 (35.8)^{b}	47.6 (44.1)^{a}	16.3 (23.8)^{e}	24.0 (29.1)^{de}	38.0 (38.0)^{c}
Btk 0.0738% + Acetamide 0.05%	4.4 (12.1)^{b}	32.4 (34.7)^{bc}	50.6 (45.3)^{a}	18.2 (25.2)^{de}	31.4 (34.0)^{b}	35.6 (36.6)^{de}
Btk 0.0950% + Benzamide 0.05%	3.0 (10.0)^{c}	28.4 (32.2)^{cd}	50.4 (45.2)^{a}	18.7 (25.6)^{de}	26.6 (31.0)^{cd}	34.4 (36.0)^{de}
C.D. (P=0.05)	(1.6)	(3.6)	(3.8)	(2.7)	(2.6)	(2.0)
SEd	0.8	1.8	1.9	1.4	1.3	1.0

Values in parentheses are transformed arc sin values.
In each column, means with similar alphabet do not vary significantly at P=0.05 by DMRT.

Table 2. Effect of *Btk* with additive chemicals (0.05%) at their LC_{50} values on weight loss of *Spodoptera litura* larvae

Treatments	% larval weight reduction over control-days after treatment				
	1st	2nd	3rd	4th	5th
$CaCO_3$ 0.05%	21.9 (27.9)^{cd}	18.1 (25.8)^{dc}	17.0 (25.3)^{f}	16.0 (23.6)^{e}	+4.0 (11.6)^{d}
$CaSO_4$ 0.05%	11.5 (19.8)^{e}	8.8 (17.2)^{f}	6.9 (15.2)^{h}	7.3 (15.6)^{f}	+3.4 (10.6)^{d}
Tween 80 0.05%	12.2 (20.4)^{e}	8.1 (16.5)^{fg}	7.2 (15.5)^{h}	7.7 (16.0)^{f}	+3.1 (10.1)^{d}
SDS 0.05%	18.9 (25.8)^{d}	14.9 (22.7)^{e}	14.1 (22.0)^{g}	15.0 (22.8)^{e}	+3.5 (10.8)^{d}
Aspartic acid 0.05%	4.4 (12.1)^{gh}	5.6 (13.6)^{fg}	5.6 (13.7)^{h}	6.9 (15.2)^{fg}	+4.0 (11.6)^{d}
Lysine 0.05%	3.9 (11.4)^{h}	5.2 (13.2)^{g}	4.7 (12.5)^{h}	6.0 (14.2)^{fg}	+3.7 (11.0)^{d}
Acetamide 0.05%	7.5 (15.8)^{f}	6.6 (14.9)^{fg}	6.2 (14.4)^{h}	6.6 (14.9)^{fg}	+3.7 (11.0)^{d}
Benzamide 0.05%	6.3 (14.6)^{fg}	6.2 (14.4)^{f}	5.5 (13.5)^{h}	4.6 12.5)^{g}	+2.4 (9.0)^{d}
Btk 0.1713%	22.8 (28.5)^{bcd}	26.6 (31.0)^{bc}	23.5 (28.9)^{e}	28.4 (32.2)^{cd}	+11.0 (19.3)^{bc}
Btk 0.0606% + $CaCO_3$ 0.05%	35.6 (36.6)^{a}	33.4 (35.3)^{a}	36.0 (36.9)^{ab}	36.6 (37.2)^{ab}	+14.0 (22.0)^{ab}
Btk 0.0854% + $CaSO_4$ 0.05%	23.6 (29.0)^{bc}	22.9 (28.5)^{cd}	25.5 (30.3)^{de}	29.2 (32.7)^{cd}	+11.6 (19.9)^{abc}
Btk 0.0728% + Tween 80 0.05%	25.6 (30.4)^{bc}	26.8 (31.2)^{bc}	27.2 (31.4)^{a}	28.0 (31.9)^{d}	+9.8 (18.2)^{c}
Btk 0.0608% + SDS 0.05%	33.4 (35.3)^{a}	35.8 (36.7)^{a}	39.6 (39.0)^{cd}	39.4 (38.9)^{a}	+14.4 (22.3)^{a}
Btk 0.0790% + Aspartic acid 0.05%	24.4 (29.6)^{bc}	30.7 (33.6)^{ab}	30.2 (33.0)^{cd}	33.0 (35.0)^{bc}	+11.3 (19.7)^{abc}
Btk 0.0700% + Lysine 0.05%	22.2 (28.1)^{cd}	33.4 (33.3)^{a}	33.6 (35.0)^{bc}	36.2 (37.0)^{a}	+12.1 (20.3)^{abc}
Btk 0.0738% + Acetamide 0.05%	27.2 (31.4)	24.8 (29.9)^{bc}	29.2 (32.7)^{cd}	31.8 (34.3)^{bc}	+12.2 (20.4)^{abc}
Btk 0.0950% + Benzamide 0.05%	26.6 (31.0)^{cd}	22.6 (28.3)^{cd}	27.2 (31.4)^{de}	30.0 (33.2)^{cd}	+10.7 (19.0)^{c}
C.D. (P=0.05)	3.2	3.8	3.2	3.1	3.0
SEd	.6	1.9	1.6	1.5	1.5

Values in parentheses are transformed arc sin values.
In each column, means with similar alphabet do not vary significantly at P=0.05 by DMRT.

Effect on Weight Loss of Larvae : The combination of treatments *Btk* + SDS resulted in significantly high % larval weight reduction on 1st day (36.6 and 39.4), 2nd day (33.4 and 35.8), 3rd day (36.0 and 39.6) and 4th day (36.6 and 39.4). *Btk* + lysine was on a par with these two treatments on 2nd day (33.4%) and on 4th day (36.2%). *Btk* + aspartic acid was on a par with these superior treatments only on 2nd day with 30.7% of larval weight reduction. In both, additive chemicals alone and

Table 3. Effect of *Btk* with additive chemicals (0.05%) at their LC_{50} values on pupation and adult emergence of *Spodoptera litura*

Treatments	% reduction over control			
	Pupal weights	Malformed pupae	Adult emergence	Malformed adults
$CaCO_3$ 0.05%	11.2 (19.5)^{c}	2.0 (8.2)^{cd}	29.0 (32.6)^{c}	0.0 (4.05)^{e}
$CaSO_4$ 0.05%	5.0 (12.7)^{d}	0.0 (4.05)^{d}	17.6 (24.8)^{d}	0.0 (4.05)^{e}
Tween 80 0.05%	5.2 (13.2)^{d}	0.0 (4.05)^{d}	16.4 (23.9)^{d}	0.0 (4.05)^{e}
SDS 0.05%	10.1 (18.5)^{c}	2.0 (8.2)^{cd}	22.0 (28.0)^{cd}	0.0 (4.05)^{e}
Aspartic acid 0.05%	4.0 (11.5)^{d}	0.0 (4.05)	17.0 (24.3)^{d}	0.0 (4.05)^{e}
Lysine 0.05%	4.7 (12.5)^{d}	2.0 (8.2)^{cd}	14.4 (22.3)^{d}	0.0 (4.05)^{e}
Acetamide 0.05%	4.7 (12.6)^{d}	4.0 (11.5)^{c}	17.6 (24.8)^{d}	0.0 (4.05)^{e}
Benzamide 0.05%	4.6 (12.4)^{d}	0.0 (4.05)^{d}	13.6 (21.6)^{d}	0.0 (4.05)^{e}
Btk 0.1713%	23.2 (28.8)^{b}	10.0 (16.3)^{ab}	54.0 (47.3)^{b}	8.0 (16.4)^{d}
Btk 0.0606% + $CaCO_3$ 0.05%	32.4 (34.9)^{a}	14.8 (22.6)^{a}	72.8 (58.7)^{a}	24.0 (29.3)^{a}
Btk 0.0854% + $CaSO_4$ 0.05%	23.6 (29.1)^{b}	12.0 (20.3)^{ab}	62.4 (52.2)^{ab}	22.0 (28.80)^{a}
Btk 0.0728% + Tween 80 0.05%	24.8 (29.8)^{b}	12.0 (20.3)^{ab}	62.4 (52.4)^{ab}	16.0 (23.6)^{bc}
Btk 0.0608% + SDS 0.05%	31.6 (34.2)^{a}	10.4 (18.0)^{ab}	70.5 (57.3)^{a}	24.0 (29.3)^{a}
Btk 0.0790% + Aspartic acid 0.05%	26.8 (31.2)^{ab}	8.0 (16.4)^{ab}	64.5 (53.4)^{ab}	16.0 (23.5)^{bc}
Btk 0.0700% + Lysine 0.05%	27.2 (31.4)^{ab}	6.4 (13.6)^{b}	64.9 (53.6)^{ab}	16.0 (23.5)^{bc}
Btk 0.0738% + Acetamide 0.05%	28.8 (32.4)^{ab}	10.0 (18.3)^{ab}	69.7 (56.50)^{a}	18.0 (25.1)^{ab}
Btk 0.0950% + Benzamide 0.05%	24.0 (29.3)^{b}	6.8 (14.4)^{b}	62.6 (52.3)^{ab}	12.0 (20.3)^{cd}
C.D. (P=0.05)	3.8	6.9	7.7	4.4
SEd	1.9	3.4	3.8	2.2

Values in parentheses are transformed arc sin values.
In each column, means with similar alphabet do not vary significantly at P=0.05 by DMRT.

their combinations with *Btk* % larval weight reduction increased over control on 5th day. For this increase (2.4 to 4.0%) the individual additive chemicals were on a par with each other. Whereas in *Btk* alone and its combinations, the increase in larval weight over control ranged between 9.8 and 14.4%. *B.t.k* combinations with $CaCO_3$, $CaSO_4$, SDS, aspartic acid, lysine and acetamide were on

par with each other in recording the larval weight increase on 5th day after the treatment (Table 2).

Effect on Weight Loss and Malformation of Pupae : Pupal weight was significantly affected by *B.t.k* in combination with additive chemicals. The effect was significantly high in *Btk* + $CaCO_3$ (32.4%) and *Btk* + SDS (31.6%). These treatments were superior to other treatments and were on par with *Btk* + acetamide (28.4%), *Btk* + lysine (27.2%) and *Btk* + aspartic acid (26.8%). *B.t.k* alone and its combination with additive chemicals at their LC_{50} values, the pupal malformity ranged from 6.4 to 14.8 %. Malformities were significantly high in *Btk* + $CaCO_3$ (14.8%), *Btk* + $CaSO_4$ (12%), *Btk* + tween 80 (12.0%), *Btk* + SDS (10.4%), *Btk* + acetamide (10.0%) *Btk* alone (10.0%) and *B.t.k* + aspartic acid (8.0%) and were on a par with each other (Table 3). Weight reduction of larvae and pupae could be attributed to the effect of larval feeding inhibition caused by *Btk* resulting in lower food intake, digestion and assimilation (Ignoffo & Gregory, 1972). Moreover, the allelochemicals present in the foliage might have interacted with the additive chemicals or *Bt* endotoxin predisposing the midgut to *Bt* protein action.

Effect on Emergence and Malformation of Adults : The descending order of reduction in adult emergence in different treatments is *B.t.k* + $CaCO_3$ (72.8%), *Btk* + SDS (70.5%), *Btk* acetamide (69.7%), *Btk* + lysine (64.9%), *B.t.k* + aspartic acid (64.5%), *Btk* + benzamide (62.6%), *B.t.k* + tween 80 (62.4%) and *Btk* + $CaSO_4$ (62.4%) and were on par with each other. The reduction in adult emergence in *Btk* alone was 54.0%. The adult malformities were significantly high in *B.t.k* + $CaCO_3$ (24.0%), *Btk* + SDS (24.0%), *Btk* + $CaSO_4$ (22.0%) and *Btk* + acetamide (18.0%) and were on par with each other. The descending order of % malformed adults in other combinations was *Btk* + aspartic acid (16.0%), *B.t.k* + lysine (6.0%), *Btk* tween 80 (16.0%), *B.t.k* + benzamide (12.0%) and *Btk* alone (8.0%) (Table 3). Malformites may be the sublethal effects of *Btk* in the left over larval populations. These effects could be attributed to difficulty in moulting and shredding of axuvia. Further, these effects might also be due to inhibition of nucleic acid and protein synthesis by *Btk* exotoxin (Ki *et al.*, 1972). Poor adults emergence might be due to weight reduction of the pupae or residual non-lethal effects in the left over populations (Dulmage & Martinez, 1973).

Thus, the potentiating effect due to inorganic salts ($CaCO_3$ and $CaSO_4$), protein solubilizing agents (tween-80 and SDS), amino acids (aspartic acid and lysine) and amides (acetamide and benzamide) was very clear with the reduction in LC_{50} values of *Btk* against *S. litura*. The effect of inorganic salts could be attributed to the increased gut pH and their action as co-factors in the proteolytic process of *Bt* (Salama *et al.*, 1986). The protein solubilizing agents might have increased the endotoxin solubility by reducing the disulphide in *Bt* protein molecules, thereby effecting the midgut epithelial cells to increase the permeability to *Bt* (Salama *et al.*, 1989). Whereas the amino acids might have effected the K^+ transport in the gut and altered the amino acid concentrations in the haemolymph thereby, potentiating the *B.t.*'s effect (Dahlman & Rosenthal, 1982).

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