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Effect of *Bacillus thuringiensis* var. *kurstaki* Kurstak with Botanicals on the Development of *Spodoptera litura* Fab.

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ABSTRACT

Botanicals, neem seed kernel extract (*Azadirachta indica* A. Juss); sweet-flag rhizome extract (*Acorus calamus* L.); pungam seed extract (*Pongamia glabra* Vent.) and annona seed extract (*Annona squamosa* L.) were evaluated for their effect on the bioefficacy of *Bacillus thuringiensis* var. *kurstaki* Kurstak (*B.t.k.*; Dipel 8L) against *Spodoptera litura* Fab. The botanicals, when used at 2.5% concentration in combination with *B.t.k.* 0.1% against *S. litura*, resulted in higher larval mortality (76.67 to 93.33%), feeding inhibition (64.69 to 90.53%) and pupal weight reduction (22.42 to 36.36%), lower larval weight gain (135.11 to 174.77 mg), pupation (6.67 to 23.33%), normal pupae (3.33 to 10.37) and normal adult emergence (0.00 to 07.78%), and prolonged larval (9.7 to 10.3 days) and pupal (11.3 to 12.0 days) periods compared to their corresponding individual effects of *B.t.k.* 0.2% and botanicals 5.0%.

Key words : Combinations, *Bacillus thuringiensis* var. *kurstaki*, *Azadirachta indica*, *Acorus calamus*, *Pongamia glabra*, *Annona squamosa*, *Spodoptera litura*.

Use of botanicals and microbial agents have been eliciting interest in the recent times as sustainable and environmentally safe pest control methods. Botanicals and bio-pesticides possess a complex of bioactive compounds, which cause different behavioural and physiological responses in insects (Khachatourians, 1986).

Bacillus thuringiensis Ber. (*B.t.)* and botanicals viz., neem (*Azadirachta indica* A. Juss), sweet-flag (*Acorus calamus* L.), pungam (*Pongamia glabra* Vent.) and annona (*Annona squamosa* L.) are such naturally occurring bio-insecticides that were proved for their efficacy against insects. Keeping in view of their low field persistence and the possibility of development of resistance against *B.t.* in insects, there is every need to analyse the scope of their integrated use. Hence, the present laboratory investigation was taken up using *B.t.* var. *kurstaki* (*B.t.k.*) with the mentioned botanicals against the tobacco caterpillar, *Spodoptera litura* Fab.

MATERIAL AND METHODS

Effect of *B.t.k.* (Dipel 8 L, Strain HD-1, Serotype-3a, 3b; M/s Sumitomo Chemical India Pvt. Ltd., Hyderabad.) and the aqueous solutions of neem seed kernel extract (NSKE: *A. indica*), sweet-flag rhizome extract (SFRE: *A. calamus*) pungam seed extract (PSE: *P. glabra*) and annona seed extract (ASE: *A. squamosa*) alone (0.2% and 5.0%, respectively) and in combination (0.1% and 2.5%, respectively) against third instar larvae of *S. litura*, reared on castor *Ricinus communis* Linn. leaves was studied in the laboratory (28 ± 2^{0}C) with three replications in completely randomized design using ten larvae per replication. The efficacy of the treatments in terms of larval mortality, feeding inhibition, larval period, weight gain, pupal weight, pupal period, pupation and adult emergence was evaluated.

Castor leaf discs dipped in different insecticidal treatments (Deshmukh and Mathai, 1991) were fed to the larvae for 24 hours and from the next day onwards fresh, clean and untreated leaves were provided as food till pupation.

The data on larval mortality was recorded and expressed as percentage. Feeding inhibition of larvae in terms of left over leaf area one day after treatment was calculated over untreated check by using leaf area meter and the per cent larval feeding inhibition was calculated by using the formula.

Per cent feeding inhibition over untreated check =

$$\frac{\text{Mean leaf area consumed in Untreated check} - \text{Mean leaf area consumed in treatment}}{\text{Mean leaf area consumed in untreated check}} \times 100$$

The mean live larval weights from one, three and five days after the treatment and the live pupal

Table1. Effect of *B.t.k.* and botanicals alone and in combination on the feeding inhibition, mortality, larval period and weight gain of *Spodoptera litura*

No.	Treatments	Per cent		Larval period after treatment (days)	Larval weight gain (mg)
		Feeding inhibition Over untreated check	Larval mortality		
1.	*B.t.k.* 0.2%	42.27 (43.43)^{g}	76.67 (61.21)^{bc}	9.0^{cde}	186.66^{e}
2.	NSKE 5.0%	75.73 (60.50)^{c}	73.33 (59.00)^{bcd}	9.3^{bcd}	193.10^{e}
3.	SFRE 5.0%	59.39 (50.42)^{e}	70.00 (56.99)^{bcd}	9.0^{cde}	210.00^{d}
4.	PSE 5.0%	41.84 (40.30)^{h}	56.67 (48.84)^{d}	8.3^{e}	235.88^{b}
5.	ASE 5.0%	52.15 (46.23)^{f}	63.33 (52.77)^{cd}	8.7^{de}	226.22^{c}
6.	*B.t.k.* 0.1% + NSKE 2.5%	90.53 (72.11)^{a}	93.33 (77.71)^{a}	10.3^{a}	135.11^{i}
7.	*B.t.k.* 0.1% + SFRE 2.5%	87.66 (69.45)^{b}	83.33 (66.14)^{b}	10.0^{ab}	149.00^{h}
8.	*B.t.k.* 0.1% + PSE 2.5%	64.69 (53.55)^{d}	76.67 (61.21)^{bc}	9.7^{abc}	174.77^{f}
9.	*B.t.k.* 0.1% + ASE 2.5%	78.38 (62.30)^{c}	80.00 (63.92)^{bc}	9.7^{abc}	166.33^{g}
10.	Untreated check	—		7.0^{f}	291.88^{a}
	F-test	Sig.	Sig.	Sig.	Sig.
	SEd.	1.11	5.58	0.36	3.14
	C.D. (0.05%)	2.33	11.73	0.76	6.61

B.t.k. : *Bacillus thrringiensis* var. *kurstaki*; **NSKE**: neem seed kernel extract;
SFRE: sweet-flag rhizome extract;
PSE: pungam seed extract; **ASE**: annona seed extract; Sig.: significant
Note: Values in each column with similar alphabet do not vary significantly

weights one day after pupation were also recorded. The larval and pupal weights were expressed as mg and per cent reduction over untreated check, respectively.

The number of total larvae entered into pupation, normal pupae and the normal adults emerged were recorded and expressed as percentage. The total time required for the larvae to enter into pupal stage after the treatment and for the pupae to emerge as adults was recorded and expressed in days as larval and pupal periods, respectively.

The data obtained were analysed by using ANOVA technique but the data in percentage were subjected to arc sine vpercentage transformation before analysis (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Larval feeding inhibition

B.t.k. 0.1% in combination with NSKE 2.5% stood first by recording 90.53 per cent larval feeding inhibition over untreated check. It was followed by other cominations of *B.t.k.* 0.1% with SFRE 2.5% (87.66%), ASE 2.5% (78.38) and PSE 2.5%

Table 2. Effect of *B.t.k.* and botanicals alone and in combination on the pupation and adult emergence of *Spodoptera litura* larva.

No.	Treatments	Per cent pupation	Per cent pupal weight reduction over untreated check	Pupal period (days)	Per cent normal pupae	Per cent normal adults
1.	*B.t.k.* 0.2%	23.33 (28.78)^{de}	19.39 (25.98)^{de}	10.7^{cd}	12.95 (21.03)^{de}	07.77 (16.09)^{ef}
2.	NSKE 5.0%	26.67 (30.99)^{cde}	17.27 (24.52)^{ef}	11.0^{bc}	11.85 (20.07)^{e}	10.37 (18.74)^{de}
3.	SFRE 5.0%	30.00 (33.21)^{cd}	16.36 (23.82)^{ef}	10.7^{cd}	16.67 (24.06)^{cd}	13.33 (21.38)^{cd}
4.	PSE 5.0%	43.33 (41.15)^{b}	09.09 (17.47)^{g}	10.0^{d}	34.66 (36.05)^{b}	24.07 (29.36)^{b}
5.	ASE 5.0%	36.67 (37.22)^{bc}	13.33 (21.36)^{f}	10.7^{cd}	19.55 (26.22)^{c}	14.39 (22.26)^{c}
6.	*B.t.k.* 0.1% + NSKE 2.5%	06.67 (12.29)^{f}	36.36 (37.07)^{a}	12.0^{a}	03.33 (10.16)^{f}	00.00 (0.00)^{h}
7.	*B.t.k.* 0.1% + SFRE 2.5%	16.67 (23.85)^{e}	30.30 (33.37)^{b}	11.7^{ab}	05.00 (12.74)^{f}	05.00 (12.76)^{g}
8.	*B.t.k.* 0.1% + PSE 2.5%	23.33 (28.78)^{de}	22.42 (28.24)^{cd}	11.3^{abc}	10.37 (18.72)^{e}	07.78 (16.12)^{ef}
9.	*B.t.k.* 0.1% + ASE 2.5%	20.00 (26.56)^{de}	25.45 (30.27)^{bc}	11.3^{abc}	10.00 (18.37)^{e}	06.67 (14.87)^{fg}
10.	Untreated check	100.00 (90.00)^{a}	0.00	09.0^{e}	100.00 (90.00)^{a}	100.00 (90.00)^{a}
	F-test	Sig.	Sig.	Sig.	Sig.	Sig.
	SEd.	3.53	1.70	0.39	1.67	1.38
	C.D. (0.05%)	7.43	3.58	0.82	3.50	2.90

B.t.k. : *Bacillus thrringiensis* var. *kurstaki*; **NSKE**: neem seed kernel extract;
SFRE: sweet-flag rhizome extract;
PSE: pungam seed extract; **ASE**: annona seed extract; Sig.: significant
Note: Values in each column with similar alphabet do not vary significantly

(64.69%). Among the individual treatments, PSE 5.0%, *B.t.k.* 0.2%, ASE 5.0%, SFRE 5.0% and NSKE 5.0% stood in the order of per cent feeding inhibition of 41.84 to 75.73 per cent. As *B.t.* activity is in the midgut, which is prime centre for food digestion and assimilation, feeding inhibition is the immediate effect (Angus, 1956).

Larval mortality

The highest per cent larval mortality of 93.33 was noticed in *B.t.k.* 0.1% + NSKE 2.5% followed by *B.t.k.* 0.1% + SFRE 2.5% (83.33%), *B.t.k.* 0.1% + ASE 2.5% (80.00%) and *B.t.k.* 0.1% + PSE (76.67%). The solo treatment, *B.t.k.* 0.2% was next to the combination treatments and resulted in 76.67 per cent larval mortality compared to 56.67 to 73.33 per cent in solo botanicals.

Larval period

In general, larval period was prolonged in combination treatment of *B.t.k.* with the botanicals. The most prolonged larval period of 10.3 days was recorded in *B.t.k.* 0.1% + NSKE 2.5%, which was on par with *B.t.k.* 0.1% + SFRE 2.5% (10.0 days) followed by *B.t.k.* 0.1% + ASE 2.5% (9.7 days) and *B.t.k.* 0.1% + PSE 2.5% (9.7 days). Even individually, *B.t.k.* 0.2% and botanicals at 5.0% prolonged the larval period (8.3 to 9.3 days) compared to the untreated check (7.0 days).

Greater prolongation of larval period in combinations is clearly a cumulative effect of the individual treatments. Feeding inhibition is one of the reasons for prolongation of larval period. The solo treatment *B.t.k.* 0.2% resulted in significantly longer larval period of 9.0 days.

Larval weight gain

Significantly the lowest mean larval weight gain (135.11 mg) was recorded in *B.t.k.* 0.1% + NSKE 2.5% followed by *B.t.k.* 0.1% + SFRE 2.5% (149.00 mg), *B.t.k.* 0.1% + ASE 2.5% (166.33 mg) and *B.t.k.* 0.1% + PSE 2.5% (174.77 mg). Significantly lower larval weight gains (186.66 to 235.88 mg) compared to the untreated check (291.88mg), but higher than the combinations were recorded in the individual treatments.

Poor larval weight gain in combinations of *B.t.k.* with the botanicals is a cumulative effect and the general trend of lower weight gain in treated *S. litura* larvae can be directly attributed to feeding deterrence. It is very characteristic of the lepidopteran larvae to attain a critical weight by voracious feeding particularly in the final instar for successful pupation. The larvae store enough food reserves and energy required for the metabolism and metamorphosis in the inactive stage of pupae (Chapman, 1968).

Poor weight gain of larvae could be directly attributed to feeding inhibition effect of *B.t.k.* resulting in lower food intake, digestion and assimilation in larvae of Lepidoptera (Ignoffo and Gregory, 1972). Moreover, the allelochemicals present in the botanicals might have interacted with *B.t.* endotoxin and reduced the larval feeding ability and predisposed them for *B.t.k.* infection (Ananthakrishnan *et al*., 1990 and Sivamani *et al*., 1992).

Pupation

Among the combinations, the lowest per cent pupation of 6.67 was recorded in *B.t.k.* 0.1% + NSKE 2.5%. It was followed by *B.t.k.* 0.1% + SFRE 2.5% (16.67%), *B.t.k.* 0.1% + ASE 2.5% (20.00%) and *B.t.k.* 0.1% + PSE 2.5% (23.33%). Because of very high larval weight reduction in these treatments, larval mortality was high, resulting in lowest number of successfully pupated larvae. The solo treatment *B.t.k.* 0.2% reduced 23.33 per cent pupation next to combination treatments compared to 26.67 to 43.33 per cent in botanicals.

Pupal period

The longest pupal period (12.0 days) was recorded in the combination, *B.t.k.* 0.1% + NSKE 2.5% followed by *B.t.k.* 0.1% + SFRE 2.5% (11.7 days). *B.t.k.* 0.1% + ASE 2.5% (11.3 days) and *B.t.k.* 0.1% + PSE 2.5% (11.3 days). Significantly prolonged pupal period of 10.0 to 11.0 days was recorded in individual treatments of *B.t.k.* 0.2% and botanicals at 5.0%.

Pupal weight

Significantly the highest per cent (36.36) pupal weight reduction over untreated check was recorded in *B.t.k.* 0.1% + NSKE 2.5% followed by *B.t.k.* 0.1% + SFRE 2.5% (30.30%), *B.t.k.* 0.1% + ASE 2.5% (25.45%) and *B.t.k.* 0.1% + PSE 2.5% (22.42%) as against 09.09 to 19.39 per cent in the individual treatments.

This variation in weight loss of the pupae might be due to decreased sequestering of larval food reserves into pupae or increased metabolic rate, which inturn might have depleted the major energy source *i.e.* fat body of the pupae.

Normal pupae

Per cent normal pupae was the lowest in *B.t.k.* 0.1% + NSKE 2.5% and *B.t.k.* 0.1% + SFRE 2.5% (3.33 and 5.00, respectively), which were on par with each other. They were followed by *B.t.k.* 0.1% + ASE 2.5% (10.00%) and *B.t.k.* 0.1% + PSE 2.5% (10.37%). Individual application of *B.t.k.* 0.2% recorded 12.95 per cent normal pupation , next to the combination treatments compared to 11.85 to 34.66 per cent in solo botanicals at 5.0%.

Normal adults

The lowest per cent normal adult emergence in the combinations is due to the cumulative effect of treatments. Among combinations, per cent adult emergence was nil in *B.t.k.* 0.1% + NSKE 2.5%. It was followed by *B.t.k.* 0.1% + SFRE 2.5% (05.00%), *B.t.k.* 0.1% + ASE 2.5% (06.67%) and *B.t.k.* 0.1% + PSE 2.5% (07.78%). But 07.77 per cent normal adult emergence was recorded in solo treatment of *B.t.k.* 0.2% as against 10.37 to 24.07 per cent in individual botanicals.

Poor adult emergence might be due to lower food reserves reflected as poor weight of pupae or due to residual non-lethal effects of the treatments in the left over population (Dulmage and Martinez, 1973).

Thus it is evident from the study that the combined use of *B.t.k.* 0.1% with botanicals at 2.5% *i.e.* at lower concentration has significant additive effect on the development of *S. litura* compared to their individual effects at higher concentrations *i.e.* *B.t.k.* 0.2% and botanicals at 5.0%.

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