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Insecticidal toxicity of commercial *Bacillus thuringiensis* (Berliner) products in combination with botanicals to *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner)

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ABSTRACT: Bioefficacy of commercial *Bacillus thuringiensis* (*B. t.*) products namely Delfin (*B. t.* var. kurstaki), Spicturin (*B. t.* var. galleriae) and Agree (*B. t.* var. kurstaki+aizawai) (each at 0.1%) in combination with botanicals *viz.*, neem (*Azadirachta indica* A. Juss) seed oil (1.5%), mint (*Mentha spicata* L.) leaf extract (2.5%), sweet flag (*Acorus calamus* L.) rhizome extract (0.5%), bracteated birthwort (*Aristolochia bracteata* Retz.) leaf extract (2.5%), mesquite (*Prosopis juliflora* DC.) leaf extract (2.5%), Indian aloe (*Aloe vira L.*) leaf extract (0.5%) and palmarosa plant (*Cymbopogan martini* Wats.) oil (0.5%) was tested in laboratory against tobacco cutworm, *Spodoptera litura* (Fabricius) and gram pod borer, *Helicoverpa armigera* (Hubner). Toxic effects of *B. t.* products and palmarosa oil in mixture were significantly superior to that of other combinations.

KEY WORDS: *Bacillus thuringiensis,* botanicals, *Helicoverpa armigera, Spodoptera litura*

Bacillus thuringiensis (Berliner) *(B. t.)* is commercially available globally under different trade names involving different *B. t.* varieties. Botanicals are other ecofriendly alternative to chemical pesticides. Farmers often have the tendency to mix botanicals with pesticides. It is therefore necessary to understand the effect of mixing of *B.t.* with botanicals. The efficacy of *B.t.* in combination with plant products like neem seed kernel extract has earlier been investigated (Hellpap and Zabitz, 1986). Salama and Sharaby (1988) studied the pathogenicity of *B. t.* in combination with powders of pomegranate peel, guava leaf and lemon grass. Balasaraswathy (1990) tried leaf extracts of *Tagetes patula* L. and *Argemone mexicana* L. in combination with *B. t.* against second instar larvae of *H. armigera*.

This paper presents the results of laboratory experiments in which mixtures of *B.t.* products and some botanicals were evaluated against *Spodoptera litura* (Fabricius) and *Helicoverpa armigera* (Hübner).

MATERIALS AND METHODS

The experiments were carried out at Agricultural College and Research Institute, Killikulam during 1996-97. The product details of *B. t.* are as follows:

The spreader, Teepol (0.5%) was added to each oil or extract. Larvae of *S. litura* were mass reared on castor (*Ricinus communis* L.) leaves at 31 ± 2°C. The disease free culture was maintained by following proper sanitary conditions. Laboratory population of *H. armigera* was established from field collected larvae and maintained by following the standard methods of Shorey and Hale (1965).

In all bioassays, leaf discs (6cm diam) of castor were used against *S. litura* and seeds of chickpea against *H. armigera*. The food materials (leaf discs or seeds) were immersed in the *B. t.* suspension for 30 seconds and the excess fluid was removed after uniform jerking before shade drying. They were then placed separately inside Petri-dishes over moist blotting paper. To each disc a single third instar larva was released after it was starved for 12 hours. For each treatment, ten larvae were used and the treatments were replicated thrice.

Commercial name	Variety / subspecies	Formulation	Spore count	Firm
Delfin	*B.t.* var. *kurstaki*	Water soluble microgranules	53,000 su/mg *S. exigua* units	Sandoz (India) Ltd.
Spicturin	*B.t.* var. *galleriae*	Suspension	1×10^6/ml	Tuticorin Alkali Chemicals
Agree	*B.t.* var. *kurstaki*	Wettable powder *aizawai*	—	Hindustan Ciba + Geigy Ltd.

The following botanicals were included:

Common name	Scientific name	Product	Conc.(%)
Neem	*Azadirachta indica* A. Juss	Seed oil	3.0
Mint	*Mentha spicata* L.	Leaf extract	5.0
Sweet flag	*Acorus calamus* L.	Rhizome extract	1.0
Bracteated birthwort	*Aristolochia bracteata* Retz.	Leaf extract	5.0
Mesquite	*Prosopis juliflora* DC.	Leaf extract	5.0
Indian aloe	*Aloe vira* L.	Leaf extract	1.0
Palmarosa	*Cymbopogan martini* Wats.	Plant oil	1.0

The larvae were allowed to feed on the treated leaf disc or seed for 24 hours. Mortality counts were made at 24 hour interval. The data in percentage were transformed into corresponding angles (Arcsine percentage) before analysis. Natural mortality was corrected (Abbott, 1925). Analysis of variance was done and means were separated by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1960; Duncan, 1955). The synergistic action of pesticidal mixtures was evaluated by comparing the observed mortality to the expected mortality (Dabi *et al.*, 1988).

RESULTS AND DISCUSSION

Laboratory bioassays revealed that *B.t.* products were significantly inferior to palmarosa oil in killing both *S. litura* and *H. armigera* larvae (Table 1). Palmarosa oil (1.0%) caused 100 per cent mortality in *S. litura* and 91.2 per cent in *H. armigera*, while *B.t.* products and other botanicals killed only 20.37 - 65.55 per cent of *S. litura* larvae and 17.48 - 47.77 per cent of *H. armigera* larvae. However, in mixture, toxicity of *B.t.* products (0.1%) and palmarosa oil (0.05 %) was significantly superior not only to *B.t.* products themselves but also to other combinations (Table 2). Palmarosa oil caused larval mortality as high as 96.29, 92.96 and 93.33 per cent in combination with Delfin, Spicturin and Agree, respectively. The larval mortality varied between 34.44 and 62.22 per cent in Delfin-botanicals assay, between 24.44 and 38.52 in Spicturin-botanicals assay, and between 30.74 and 51.48 per cent in Agree-botanicals assay. Independently, Delfin, Spicturin and Agree caused only 53.33, 35.18 and 65.55 per cent mortality, respectively in *S. litura*.

Table 1. Efficacy of *B. t.* and botanicals against *S. litura* and *H. armigera*

Treatment	Concentration (%)	Per cent larval mortality at 72 h			
		H. armigera		*S. litura*	
Delfin	0.2	47.77	(43.72)^{bc}	53.33	(46.06)^{bc}
Spicturin	0.2	34.50	(35.73)^{c}	35.18	(36.12)^{cd}
Agree	0.2	41.29	(39.88)^{bc}	65.55	(54.10)^{b}
Neem (oil)	3.0	34.96	(36.18)^{c}	38.10	(38.08)^{cd}
M. spicata (leaf extract)	5.0	28.10	(31.99)^{cd}	27.40	(31.31)^{cd}
A. calamus (rhizome extract)	1.0	29.42	(32.52)^{cd}	57.77	(32.56)^{cd}
A. bracteata (leaf extract)	5.0	17.48	(24.69)^{d}	20.37	(26.41)^{d}
P. juliflora (leaf extract)	5.0	31.20	(33.85)^{cd}	34.07	(35.33)^{cd}
A. vira (leaf extract)	1.0	54.02	(47.35)^{b}	51.11	(45.68)^{bc}
Palmarosa (oil)	1.0	91.20	(73.34)^{a}	100.00	(90.00)^{a}
Control		5.00	(4.31)^{e}	3.33	(6.15)^{e}

Figures in parentheses are angular transformed values. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

Table 2. Toxicity of *B. t.* + botanical to *S. litura*

Treatment	Concentration (%)	Per cent larval mortality at 72 h					
		Delfin		Spicturin		Agree	
B.t. alone	0.2	53.33	(46.06)^{bc}	35.18	(36.12)^{b}	65.55	(54.10)^{b}
B.t. + neem oil	0.1+1.5	62.22	(52.09)^{b}	32.22	(34.38)^{b}	44.81	(42.01)^{bc}
B.t. + *M. spicata*	0.1+2.5	55.18	(47.99)^{bc}	28.15	(31.83)^{b}	34.81	(54.10)^{b}
B.t. + *A. calamus*	0.1+0.5	51.11	(45.69)^{bc}	38.52	(38.05)^{b}	51.48	(43.94)^{bc}
B.t. + *A. bracteata*	0.1 +2.5	34.44	(35.90)^{c}	25.18	(29.98)^{b}	37.40	(37.45)^{c}
B.t. + *P. juliflora*	0.1+2.5	51.48	(45.94)^{bc}	32.59	(34.55)^{b}	33.04	(32.56)^{c}
B.t. + *A. vira*	0.1+0.5	44.81	(42.01)^{bc}	24.44	(28.94)^{b}	30.74	(33.52)^{c}
B.t. + palmarosa oil	0.1+0.5	96.29	(83.51)^{a}	92.96	(77.36)^{a}	93.33	(77.71)^{a}
Control	–	3.33	(6.15)^{d}	6.67	(12.29)^{c}	3.33	(6.15)^{d}

Figures in parentheses are angular transformed values. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

The response of *B. t.* + botanical mixtures was either synergistic or antagonistic (Table 3). Palmarosa oil (1%) was positively synergistic with Delfin, Spicturin and Agree, while extracts of *A. calamus* (0.5%), *A. bracteata* (2.5%) and *A.vira* (0.5%) were antagonistic. The response from neem oil (1.5%) and from extracts of *M. spicata* (2.5%) and *P. juliflora* (2.5%) was synergistic with Delfin but antagonistic with Spicturin and Agree, probably due to variations in product additives.

The efficacy of *B. t.* products in mixture with botanicals differed significantly against *H. armigera* (Table 4). However, the effect of palmarosa oil alone was significant in improving the toxicity of each *B.t.* product. When Delfin was tried in combination with botanicals, the mortality from *B.t.* + palmarosa oil was highest (96.48). The mortality varied between 29.81 per cent in *B.t.* + *A . bracteata* and 55.18 per cent in *B.t.* + neem oil. Similarly, Spicturin caused as high as 93.07 per cent mortality of the larvae in combination with palmarosa oil. Other treatments induced low mortality ranging between 27.63 per cent from Spicturin + *A. bracteata* and 39.56 per cent from Spicturin + neem oil. Mortality from Agree + palmarosa oil was significantly highest in *H. armigera* (94.81 %). Rest of the treatments were inferior and the larval mortaiity in these treatments varied from 34.26 to 49.81 per cent.

Table 3. Synergistic response of *B. t.* products + botanical to *S. litura*

Treatment	Concentration (%)	Larval mortality (%)		Synergistic response
		Observed	Expected	
Delfin + neem oil	0.1+1.5	62.22	45.71	Positive
Delfin + *M. spicata*	0.1+2.5	55.18	40.37	Positive
Delfin + *A. calamus*	0.1+0.5	51.11	55.55	Negative
Delfin + *A. bracteata*	0.1+2.5	34.44	36.85	Negative
Delfin + *P. juliflora*	0.1+2.5	51.48	43.70	Positive
Delfin + *A. vira*	0.1+0.5	42.81	52.22	Negative
Delfin + palmarosa oil	0.1+0.5	96.29	76.66	Positive
Spicturin + neem oil	0.1+1.5	32.22	36.64	Negative
Spicturin + *M. spicata*	0.1+2.5	28.15	31.29	Negative
Spicturin + *A. calamus*	0.1+0.5	38.52	46.47	Negative
Spicturin + *A. bracteata*	0.1+2.5	25.18	27.77	Negative
Spicturin + *P. juliflora*	0.1+2.5	32.59	34.62	Negative
Spicturin + *A. vira*	0.1+0.5	24.44	43.15	Negative
Spicturin + palmarosa oil	0.1+0.5	92.96	67.59	Positive
Agree + neem oil	0.1+1.5	44.81	51.82	Negative
Agree + *M. spicata*	0.1+2.5	34.81	46.48	Negative
Agree + *A. calamus*	0.1+0.5	51.48	61.16	Negative
Agree + *A. bracteata*	0.1+2.5	37.40	42.96	Negative
Agree + *P. juliflora*	0.1+2.5	33.04	49.81	Negative
Agree + *A. vira*	0.1+0.5	30.74	58.33	Negative
Agree + palmarosa oil	0.1+0.5	93.33	82.77	Positive

Table 4. Toxicity of *B. t.* + botanical to *H. armigera*

Treatment	Concentration (%)	Per cent larval mortality at 72 h					
		Delfin		Spicturin		Agree	
B.t. alone	0.2	47.77	(43.72)^{bc}	34.30	(35.73)b	41.29	(39.88)b
B.t. + neem oil	0.1+1.5	55.18	(47.99)^{b}	39.56	(38.93)^{b}	49.81	(44.89)^{b}
B.t. + *M. spicata*	0.1+2.5	55.00	(47.91)^{b}	30.96	(33.65)^{b}	36.29	(36.83)^{b}
B.t. + *A. calamus*	0.1+0.5	46.48	(42.98)^{bc}	34.56	(35.95)^{b}	44.63	(41.89)^{b}
B.t. + *A. bracteata*	0.1 +2.5	29.81	(32.67)^{c}	27.63	(31.69)^{b}	34.26	(35.71)^{b}
B.t. + *P. juliflora*	0.1+2.5	39.07	(38.42)^{bc}	36.05	(36.84)^{b}	40.92	(37.72)^{b}
B.t. + *A. vira*	0.1+0.5	48.15	(43.94)^{bc}	39.65	(40.96)b	35.74	(36.49)^{b}
B.t. + palmarosa oil	0.1+0.5	96.48	(82.15)^{a}	93.07	(77.89)^{a}	94.81	(82.26)^{a}
Control		6.67	(12.29)^{d}	3.33	(6.15)^{c}	3.33	(6.15)^{c}

Figures in parentheses are angular transformed values. In a column, means followed by a common letter are not significantly different at 5% level by DMRT.

The response against *H. armigera* of *B.t.* + botanical mixtures was either synergistic or antagonistic (Table 5). The response of mixture containing *B.t.* products + either neem oil or extract of *M. spicata* and palmarosa oil was always synergistic. The response from *B.t.* + extract of *A. vira* was invariably antagonistic. Extracts of *A. calamus*, *A. bracteata* and *P. juliflora* in combination with *B. t.* products responded either way. Thus *A. calamus* was synergistic with Delfin but antagonistic with Spicturin and Agree. *A. bracteata* and *P. juliflora* were antagonistic with Delfin but synergistic with Spicturin and Agree, probably because of the adjuvants involved in each product.

As botanical preparations and microbial pathogens are fast replacing synthetic insecticides, increasing tendency among farmers to mix them up will help the prospects of biocontrol. Results of these experiments provide a better insight into the scope of mixing biopesticides with botanicals. Earlier formulations containing viable spores of *B. t.* and sublethal doses of insecticides have been reported to be more effective (Mohamed *et al.*, 1983; Justin *et al.*, 1989; Zaz and Kushwaha, 1993). Such combinations have been either synergistic or antagonistic. Plamarosa oil alone was invariably outstanding in improving the effectiveness of each *B.t.* product against both the larvae. Extracts of *A. calamus*, *A. bracteata* and *A. vira* often suppressed the insecticidal effects of *B. t.* against *S. litura*. Other botanicals proved rather inconsistent. Neem oil as well as extracts of *M. spicata* and of *P. juliflora*

was synergistic with Delfin but antagonistic with Spicturin and Agree when tested against *S. litura*. On the other hand, neem oil and *M. spicata* extract showed synergism against *H. armigera*, while other combinations had mixed effects. Hellpap and Zabitz (1986) reported that *B. t. k.* (Dipel 0.1 %) caused high mortality in *S. frugiperda* even at lower doses when used in combination with neem seed kernel extract. The results indicate that oils of palmarosa and neem merit consideration while preparing *B.t.* + botanical mixtures.

Table 5. Synergistic response of *B. t.* products + botanical against *H. armigera*

Treatment	Concentration (%)	Larval mortality (%)		Synergistic
		Observed	Expected	
Delfin + neem oil	0.1+1.5	55.18	41.36	Positive
Delfin + *M. spicata*	0.1+2.5	55.00	37.93	Positive
Delfin + *A. calamus*	0.1+0.5	46.48	38.59	Positive
Delfin + *A. bracteata*	0.1+2.5	29.81	32.63	Negative
Delfin + *P. juliflora*	0.1+2.5	31.07	39.49	Negative
Delfin + *A. vira*	0.1+0.5	48.15	50.89	Negative
Delfin + palmarosa oil	0.1+0.5	96.48	69.48	Positive
Spicturin + neem oil	0.1+1.5	39.56	36.12	Positive
Spicturin + *M. spicata*	0.1+2.5	30.96	30.76	Positive
Spicturin + *A. calamus*	0.1+0.5	34.56	45.95	Negative
Spicturin + *A. bracteata*	0.1+2.5	27.63	27.25	Positive
Spicturin + *P. juliflora*	0.1+2.5	36.05	34.10	Positive
Spicturin + *A. vira*	0.1+0.5	39.65	42.62	Negative
Spicturin + palmarosa oil	0.1+0.5	93.07	67.06	Positive
Agree + neem oil	0.1+1.5	49.81	39.69	Positive
Agree + *M. spicata*	0.1+2.5	36.29	34.35	Positive
Agree + *A. calamus*	0.1+0.5	44.63	49.53	Negative
Agree + *A. bracteata*	0.1+2.5	34.26	30.83	Positive
Agree + *P. juliflora*	0.1+2.5	40.92	37.68	Positive
Agree + *A. vira*	0.1+0.5	35.74	46.20	Negative
Agree + palmarosa oil	0.1+0.5	94.81	70.65	Positive

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