Comparative efficacy of plant extracts alone and in combination with *Bacillus thuringiensis* sub sp. *kurstaki* against *Spodoptera litura* Fab. larvae

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ABSTRACT

Efficacy of crude aqueous extracts of eight plant species was assessed against *Spodoptera litura* larvae at 25, 50, 75 and 100 % concentrations alone and in combination with *Bacillus thuringiensis* sub sp. *kurstaki* Berliner (Btk) under controlled laboratory conditions. Leaf extracts of *Acacia arabica* and *Annona squamosa* were found most promising causing 76.66 % and 83.33 % larval mortality respectively at 25 % concentration within 3 days of treatment. Leaf extract of *Datura stramonium* inflicted 93.33 % larval mortality at 50 % concentration within 4 days. When used in combination with Btk, increased larval mortality could be observed *vis-à-vis* plant extracts alone in cases of seed extracts of *A. arabica*, *A. squamosa* and *D. stramonium* and leaf extract of *Pongamia pinnata* only on 3 days of treatment and onwards indicating synergistic effect. However, leaf extracts of *A. arabica*, *A. squamosa*, *D. stramonium*, *Eucalyptus globulus*, *Ipomoea carnea*, *Lantana camara* and *Nicotiana tabacum* had antagonistic effect when used in combination with Btk.

Key words: *Bacillus thuringiensis*, efficacy, plant extracts, soybean, *Spodoptera litura*, synergism

INTRODUCTION

Bacillus thuringiensis (Bt) based microbial insecticides have proved to be effective against lepidopteran defoliators (Sharma, 2000; Sharma and Ansari, 2004) and provide an environmentally safe and sustainable alternative to largely hazardous, broad spectrum and toxic chemical insecticides. On the other hand, several plant species have been reported to possess insecticidal properties (Raheza, 1998; Singh, 2000). They are also responsible for affecting the food consumption and utilization by the insects (Rajguru *et al*., 2010). Nevertheless, continuous use of any plant protection strategy is bound to pose certain unwarranted consequences, the most important being development of insecticides resistance in insects. In order to avoid or delay this process, combination of microbial pesticides with insecticidal compounds of plant origin consisting of diverse compounds with novel and different modes of action can help in reducing the risk and also probability of simultaneous resistance development through the synergism (Regev *et al*., 1996; Khanuja *et al*, 2002). The present investigation was, therefore, carried out to compare the efficacy of some plant extracts alone as well as in combination *Bacillus thuringiensis* against *Spodoptera litura* larvae, which has become one of the major insect-pests threatening soybean cultivation in many Indian states *viz.*, Madhya Pradesh, Rajasthan and Maharashtra.

MATERIALS AND METHODS

A laboratory experiment was conducted at Directorate of Soybean Research, Indore during 2009-10, using combinations of 11 plant extracts from eight popular and commonly available plant species and *Bacillus thuringiensis* sub sp *kurstaki* (Btk) strain HD-1 (DiPel 8 L; 17600 IU/g, by M/s Valent Bio-Sciences Corporation, USA). The stock solution of extracts was prepared by grinding 100 g of plant material (leaves/seeds) with 500 ml distilled water and was filtered through Whatman No. 42 filter paper. This was diluted to obtain different concentrations *viz.*, 25

%, 50%, 75% and 100%. Btk was used in recommended dose of 1.0 lit /ha applied through 750 lit of water (Sharma, 2000). Accordingly, in all the plant extracts Btk was added at the rate 0.026 ml per 20 ml of extract to make the desired combination. The culture of *S. litura* was developed from larvae collected from the Soybean fields of Directorate of Soybean Research, Indore and was maintained in the entomological chamber.

To compare the efficacy of plant extracts alone or in combination with Btk, ten pre starved (3 hrs) 2nd instar *S. litura* larvae were released in petri-plates (6 inch diameter) having fresh soybean leaves. The leaves and the larvae of one set were sprayed with different concentrations of plant extracts alone and the other with combination of plant extracts and Btk with the help of hand sprayer. Three replications were maintained for each concentration and treatment. Entire experimental set was kept in Entomological Chamber (Spectrum make) maintained at 75 ± 5% RH, 26 ± 1^0C temperature and 12 hrs photophase and scotophase. Observations on larval mortality were recorded every 24 hrs up to 6 days of treatment.

RESULTS

Efficacy of treatments varied with concentration and time. Larval mortality increased with increase in concentration and passage of time. Initially i.e. 1 day after exposure, it was observed that plant extracts alone caused significantly higher larval mortality than the corresponding extracts with Btk at all concentration (Table 1). Extracts with Btk at 25% and 50% concentration did not cause any larval mortality except for *A. squamosa* seed and *D. stramonium* seed extracts even up to 2 days of exposure. This could be due to inadequate ingestion of treated food material by the larvae. Moreover, it is well known that entomopathogenic bacteria undergo a log-phase

Table 1. Comparison of per cent larval mortality with different concentrations of *A. Arabica, A. squamosa, D. Stramonium,* and *E. globulus* applied (PE) alone and in combination with *Bacillus thuringiensis* (Bt) (PE+Bt) after 1 to 6 days of exposure

Treatment	Days	Concentrations (%)							
		25		50		75		100	
		PE	PE+Bt	PE	PE+Bt	PE	PE+Bt	PE	PE+Bt
A. arabica	1	36.66	0.00	46.66	0.00	50.00	0.00	70.00	0.00
	2	66.66	0.00	66.66	0.00	70.00	0.00	70.00	0.00
	3	76.66	20.00	80.00	20.00	86.66	20.00	86.66	26.66
	4	86.66	26.66	86.66	26.66	86.66	33.33	86.66	40.00
	5	93.33	26.66	100.00	33.33	100.00	33.33	100.00	40.00
	6	100.00	26.66	100.00	40.00	100.00	40.00	100.00	46.66
A. squamosa	1	43.33	0.00	50.00	0.00	60.00	6.66	60.00	13.33
	2	60.00	0.00	70.00	0.00	70.00	13.33	70.00	13.33
	3	83.33	6.66	86.66	33.33	93.33	46.66	93.33	73.33
	4	83.33	40.00	86.66	46.66	93.33	73.33	100.00	80.00
	5	100.00	46.66	100.00	46.66	100.00	73.33	100.00	86.66
	6	100.00	53.33	100.00	53.33	100.00	73.33	100.00	86.66
D. stramonium	1	36.66	0.00	76.66	0.00	76.66	0.00	86.66	0.00
	2	43.33	0.00	86.66	6.66	86.66	6.66	100.00	6.66
	3	60.00	0.00	86.66	13.33	93.33	26.66	100.00	26.66
	4	76.66	46.66	93.33	60.00	93.33	66.66	100.00	86.66
	5	93.33	53.33	100.00	60.00	100.00	66.66	100.00	80.00
	6	93.33	60.00	100.00	60.00	100.00	66.66	100.00	80.00
E. globulus	1	26.66	0.00	43.33	0.00	46.66	0.00	53.33	0.00
	2	30.00	0.00	60.00	0.00	66.66	6.66	70.00	13.33
	3	43.33	13.33	66.66	13.33	70.00	26.66	76.66	66.66
	4	43.33	20.00	76.66	20.00	76.66	53.33	76.66	66.66
	5	76.66	20.00	83.33	20.00	93.33	66.66	93.33	73.33
	6	83.33	20.00	86.66	33.33	93.33	66.66	93.33	73.33

Table 2. Comparison of per cent larval mortality with different concentrations of *I. Carnea*, *L. camara*, *N. tabacum* and *P. pinnata* applied (PE) alone and in combination with *Bacillus thuringiensis* (Bt) (PE+Bt) after 1 to 6 days of exposure

Treatment	Days	Concentrations (%)							
		25		50		75		100	
		PE	PE+Bt	PE	PE+Bt	PE	PE+Bt	PE	PE+Bt
I. carnea	1	26.66	0.00	26.66	0.00	26.66	13.33	60.00	26.66
\	2	33.33	0.00	60.00	6.66	66.66	33.33	83.33	40.00
	3	53.33	33.33	70.00	33.33	83.33	73.33	86.66	93.33
	4	53.33	40.00	70.00	46.66	86.66	73.33	100.00	93.33
	5	76.66	60.00	86.66	60.00	100.00	73.33	100.00	93.33
	6	76.66	66.66	93.33	60.00	100.00	73.33	100.00	93.33
L. camara	1	16.66	0.00	36.66	0.00	50.00	0.00	53.33	13.33
	2	26.66	0.00	50.00	0.00	60.00	13.33	60.00	13.33
	3	50.00	26.66	60.00	60.00	60.00	60.00	76.66	73.33
	4	50.00	40.00	60.00	60.00	76.66	66.66	80.00	73.33
	5	76.66	60.00	76.66	60.00	76.66	66.66	100.00	73.33
	6	76.66	60.00	83.33	60.00	86.66	66.66	100.00	73.33
N. tabacum	1	30.00	0.00	53.33	0.00	53.33	6.66	60.00	13.33
	2	30.00	0.00	70.00	6.66	70.00	20.00	86.66	26.66
	3	53.33	46.66	76.66	66.66	80.00	86.66	86.66	86.66
	4	53.33	46.66	76.66	46.66	80.00	86.66	86.66	100.00
	5	53.33	46.66	76.66	86.66	86.66	93.33	86.66	100.00
	6	60.00	60.00	83.33	86.66	86.66	93.33	93.33	100.00
P. pinnata	1	0.00	0.00	0.00	0.00	10.00	6.66	10.00	6.66
	2	0.00	0.00	16.66	6.66	16.66	6.66	20.00	20.00
	3	16.66	26.66	16.66	26.66	26.66	53.33	43.33	86.66
	4	16.66	26.66	26.66	53.33	36.66	66.66	76.66	86.66
	5	33.33	26.66	53.33	70.00	66.66	70.00	76.66	86.66
	6	33.33	26.66	76.66	60.00	86.66	66.66	93.33	86.66

to reach to toxic levels. Highest mortality was exhibited by 25% leaf extract of *A. squamosa* alone which was *on par* with that observed with many extracts applied with or without Btk even at higher concentrations. *Datura stramonium* leaf extract also showed appreciable mortality but required 50% concentration which was *on par* with its higher concentrations. After 2 days of exposure the larval mortality caused by 25% leaf extracts of *A. arabica*, *A. squamosa* and *Datura stramonium* was *on par* with their respective higher concentrations (Table 1). When seed extracts of *A. arabica*, *A. squamosa* and *Datura stramonium* were considered, they were found significantly more effective when mixed with Btk than their respective extracts alone after 3 days of exposure, showing synergistic effect (Table 2). On the other hand when Btk was mixed in leaf extracts of these plants, the efficacy of combination was reduced as compared to leaf extracts alone, exhibiting antagonistic effect. The differences between the mortality due to seed extracts of *A. arabica*, *A. squamosa* and *Datura stramonium* with Btk and without Btk were significant and more conspicuous on 3rd and 4th day (Tables 3 and 4). 100% larval mortality could be achieved with 100% concentration of *D. stramonium* leaf extract alone and *A. arabica* seed extract fortified with Btk. As the time progressed, higher mortality (more than 90%) was exhibited by some extracts even at lower concentrations *viz.*, leaf extracts of *A. arabica*, *A. squamosa* and *Datura stramonium* at 25 and 50% concentrations. However, seed extracts of *A. arabica* and *A. squamosa* showed 100% larval mortality when fortified with Btk only at 75% and 100% concentrations (Tables 3). It was evident that leaf extracts of *A. arabica*, *A. squamosa*, *D. stramonium*, *E. globulus*, *I. carnea*, *L. camara* and *N. tabacum* were able to cause considerable larval mortality without Btk right from the lower doses. These extracts when used in combination with Btk showed reduced larval mortality indicating antagonistic effect, the most conspicuous being in

Table 3. Comparison of per cent larval mortality with different concentrations of *A. arabica* , *A. squamosa* and *D. stramonium* seed applied (PE) alone and in combination with *Bacillus thuringiensis* (Bt) (PE+Bt) after 1 to 6 days of exposure

Treatment	Days	Concentrations (%)							
		25 %		50 %		75 %		100 %	
		PE	PE+Bt	PE	PE+Bt	PE	PE+Bt	PE	PE+Bt
A. arabica (seeds)	1	0.00	0.00	10.00	0.00	10.00	0.00	20.00	0.00
	2	16.66	6.66	20.00	6.66	20.00	6.66	33.33	13.33
	3	20.00	73.33	33.33	86.66	36.66	86.66	43.33	100.00
	4	36.66	73.33	50.00	80.00	53.33	86.66	63.33	100.00
	5	60.00	80.00	63.33	86.66	86.66	100.00	86.66	100.00
	6	63.33	80.00	70.00	86.66	86.66	100.00	100.00	100.00
A. squamosa (seeds)	1	26.66	6.66	36.66	20.00	46.66	40.00	70.00	40.00
	2	36.66	33.33	36.66	46.66	53.33	73.33	70.00	86.66
	3	36.66	53.33	43.33	86.66	53.33	93.33	70.00	93.33
	4	43.33	80.00	53.33	93.33	70.00	100.00	76.66	100.00
	5	43.33	80.00	53.33	93.33	70.00	100.00	76.66	100.00
	6	53.33	80.00	76.66	93.33	70.00	100.00	80.00	100.00
D. stramonium (seeds)	1	10.00	6.66	10.00	6.66	16.66	20.00	26.66	26.66
	2	26.66	13.33	26.66	13.33	36.66	26.66	36.66	53.33
	3	26.66	40.00	36.66	53.33	53.33	73.33	63.33	86.66
	4	26.66	60.00	53.33	60.00	60.00	73.33	63.33	93.33
	5	53.33	76.66	60.00	76.66	73.33	80.00	86.66	93.33
	6	53.33	76.66	60.00	80.00	83.33	86.66	93.33	93.33

case of *Acacia* leaf extract and Btk combination. However, the efficacy of seed extracts of *A. arabica*, *A. squamosa* and *D. stramonium* was better when used in combination with Btk, exhibiting synergistic action. This differential effect of different plant parts could be attributed to differences in their chemical constituents and their interaction with Btk.

DISCUSSION

In earlier studies on similar aspects *D. stramonium* seed extract fortified with *Bacillus thuringiensis* recorded as high as 74.7% mean larval mortality (Rajguru *et al*., 2011a). Using ethanol for extraction of active principals was reported to be more effective, as the ethanol extracts required less concentration for appreciable larval mortality (Rajguru *et al*., 2011b). Babu *et al.* (1998) reported about 60% mortality in *S. litura* larvae with 5% concentration of *A. squamosa* crude oil. They also reported seed extract of *A. squamosa* to be highly toxic even to 5th instar larvae (Babu *et al*., 1999). Fifty per cent larval mortality in fourth instar larvae of *S. litura* was observed by Behera and Satapathy (1996) when they used 10 % concentration of aqueous extract of *P. pinnata*.

Antifeedant and toxic properties of several plant extracts against different insects have been assessed by various workers – *I. carnea* against *Achaea janata* (Arivudainambi and Nachiappan 1993); *Nicotiana tabacum* and *Datura metal* against *S. litura* (Murugan *et al.,* 1998); *D. stromonium* against *Manduca sexta* (Katanyukul and Thurston, 1979); *Eucalyptus* spp. against *Plutella xylostella* (Dwivedi and Mathur, 1999) and against *Plecoptera reflexa* (Meshram, 2000). In another study, neem oil and *Pongamia* oil were found to have synergistic effect on the mortality of *Culex quinquefasciatus* when used in combination with *Bacillus thuringiensis israelensis* (Muruean *et al*., 2002).

It is clear from the above discussion that most of the earlier studies were aimed at separate assessment of efficacy of either aqueous extracts or ethanol extracts or the synergistic effect of combination of plant extracts and insect pathogens. Results of this study provided useful information as to which plant extracts can be used alone and which in combination with Btk. Above comparison clearly established that combination of *A. arabica* seed, *A. squamosa* seed, *D. stramonium* seed and *N. tabacum* leaf extracts and Btk had higher

efficacy against *S. litura* larvae than the respective extracts without Btk. However, combination of Btk with remaining plant extracts appeared to have antagonistic effect, as in these cases the plant extracts alone exhibited higher larval mortality as compared to their combinations with Btk.

Hence, it can be concluded that leaf extracts of *A. arabica*, *A. squamosa*, *D. stramonium*, *E. globulus*, *L. camara* and *P. pinnata* are effective against *S. litura* larvae causing significant mortality when used alone, while extracts of *N. tabacum*, *A. arabica* seed, *A. squamosa* seed and *D. stramonium* seed are more effective when fortified with *Bacillus thuringiensis*. The extracts found to be effective as standalone and / or in combination with Bt are being further validated through testing under field conditions.

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