In vitro study on the effect of bhendi varieties on the infectivity of *Beauveria bassiana* (Bals.) Vuill to *Spodoptera litura* Fab

T. Suganya and V. Selvanarayanan*

ABSTRACT

Laboratory experiments on the evaluation of infectivity of *Beauveria bassiana* (Bals.) Vuill. to tobacco caterpillar *Spodoptera litura* Fab. as influenced by two okra varieties. Infectivity of *B. bassiana* at three concentrations *viz*. 0.1, 1.0 and 3.0 per cent was evaluated by contaminated food bioassay method. In comparison, topical bioassay method was also made. Statistically significant variation between the two okra varieties was observed with regard to the mortality of *S. litura*. When the leaf extract of the bhendi variety P 7 was amended to the culture medium of *B. bassiana*, the maximum conidial germination, sporulation and mycelial growth were recorded as against the minimum with the variety Pusa Sawani. Hence, it is concluded that plant factors influence infectivity of the entomopathogenic fungi *B. bassiana*.

Key words: Entomopathogenic fungi, tri-trophic interaction, host plants, crop pest

INTRODUCTION

Use of Entomo-Pathogenic Fungi (EPF) against major pests in various crop ecosystems is being popularized and many such EPF are commercially exploited as Mycoinsecticides. Relatively less attention is being paid to unravel the third trophic level influence of the plant-derived compounds on infectivity of EPF. Plant chemistry is considered as an important component of tri-trophic relationships between plants, insects and microbial control agents (Inyang *et al.*, 1999a).

The efficacy of entomopathogenic fungi is largely dependent upon sufficient amounts of viable, virulent inoculum adhering to the surface of the target host. Many foliar insects acquire spores when they walk or crawl over inoculated leaf surfaces. In such circumstances, leaf topography can influence spore acquisition but stimulatory or inhibitory compounds on the phylloplane could affect the infection process (Inyang *et al.*, 1999b). Many of the plant biochemical components such as major nutrients, minor elements, epicuticular compounds, phenols, alkaloids *etc*. influence the activity of entomopathogenic fungi. The alkaloids tomatine and solanine in solanaceous plants (Costa and Gaugler, 1989a, b; Gallardo *et al.*, 1990; Lacey and Mercadier, 1998; Poprawski *et al.*, 2000), gossypol in cotton (Poprawski and Jones, 2001); lignin related phenolic compounds (Guirad *et al.*, 1995), epicuticular waxes (Inyang *et al.*, 1999a), isothiocyanates (Inyang *et al*., 1999b; Klingen *et al*., 2002), tannic, gallic and chlorogenic acid (Vega *et al*., 1997) and other biochemicals are reported to influence EPF. Because of the presence of such host specific biochemicals, variation in the virulence of *B. bassiana* has become regular phenomena witnessed in different crop ecosystems, even with regard to same pest species (Pena *et al.*, 1995; Poprawski *et al.*, 2000; Poprawski and Jones, 2001; Kreutz *et al.*, 2004; Anitha Bharath, 2005; Alvarez *et al.*, 2006 and Karthikeyan, 2006). In contrast to the above, Klingen *et al*. (2002) and Cuthbertson *et al*. (2005) observed no significant influence of host plants on the survival and infectivity of EPF.

Insect resistance in crop plants manifested in the form of antibiosis is generally mediated through biochemical factors. Many such biochemicals besides conferring insect resistance, influences the activity of natural enemies including insect pathogenic microorganisms, as reviewed above. This concept formed the basis for the present study to analyse the influence of host plant factors in bhendi on the pathogenicity of *Beauveria bassiana* (Bals.) Vuill. to *Spodoptera litura* Fab.

MATERIALS AND METHODS

Beauveria bassiana (Beevicide® from Romvijay Biotech Private Limited, Puducherry) was evaluated for its interaction with leaf caterpillar, *S. litura* as influenced by bhendi varieties. The test insect, *S. litura* was mass cultured in the laboratory on standard artificial diet and used for the study. Bhendi varieties namely P_7 and Pusa Sawani reported to be insect resistant and susceptible varieties respectively (Kannan, 1995) were selected for

Table 1. Bioassay of *B. bassiana* against *S. litura* feeding on tolerant and susceptible bhendi varieties

Bhendi varieties	Mortality (%) of *S. litura*				
	Concentration of *B. bassiana* (in %)				
	0.1	1	3	Control	Mean
	Topical bioassay				
P 7	68.00 (55.83)	80.00 (66.21)	84.00 (71.52)	0.00 (0.00)	58.00
Pusa Sawani	36.00 (36.47)	60.00 (51.22)	68.00 (55.83)	0.00 (0.00)	41.00
Mean	46.15	58.71	63.68	0.00	
	Between Varieties	Among Trt.	Varieties X Trt.		
C.D.	5.89	8.33	11.78		
Contaminated food bioassay					
P 7	28.00 (28.62)	44.00 (41.31)	64.00 (53.52)	0.00 (0.00)	34.00
Pusa Sawani	24.00 (23.31)	36.00 (36.47)	48.00 (43.84)	0.00 (0.00)	27.00
Mean	25.97	38.89	48.68	0.00	
	Between Varieties	Among Trt.	Varieties X Trt.		
C.D.	8.47	11.99	16.95		

Values in parentheses are arcsine transformed

the study. For pot culture studies, the test plants @ one plant per pot were maintained in earthen pots of 30 cm height and 30 cm diameter. The pots were filled with potting mixture comprising two parts of soil, one part of red earth and one part of FYM.

The efficacy of *B. bassiana* against *S. litura* was evaluated by two methods of bioassay *viz.*, topical and contaminated food bioassay. Three concentrations of *B. bassiana viz.*, 0.1, 1.0 and 3.0 per cent were evaluated in comparison with a control wherein only sterile water was used. Five replications were maintained per treatment with five insects per replication. For topical bioassay, uniform aged test insects taken in a Petri plate (8 cm) lined with a sterilized filter paper were sprayed directly with the spore suspension using hand atomizer. For contaminated food bioassay, the spore suspension was applied to both the upper and lower surfaces of leaf discs excised from the respective variety. After air drying, the treated insects were carefully transferred to individual sterile plastic containers having the respective food source already washed with sterile distilled water. The treated insects were kept undisturbed in Petri plates and observations on the development of fungal mycelia and subsequent mortality were recorded on seventh day after treatment.

For evaluating the influence of leaf extracts of bhendi varieties on the growth of *B. bassiana*, fresh leaves of the varieties were processed with sterile distilled water @ 1 ml per g of tissue (1:1 v/w) with pestle and mortar and filtered through a double layered cheese cloth to prepare the standard leaf extract solution (100%). Potato Dextrose Agar medium was prepared in 250 ml Erlenmeyer flasks and 5, 20 and 40 ml of leaf extracts were added to 45, 30 and 10 ml aliquots respectively in the flask so as to get the final concentration of 10, 40 and 80 per cent of the extracts in the medium. The medium without any extract served as control. The medium was poured into 90 mm Petri plates at the rate of 15 ml/plate. The culture discs of nine mm size obtained from a seven days old culture of *B. bassiana* were taken and inoculated in the centre of the Petri plates aseptically and incubated for seven days. The diameter of the colony was measured after the mycelium fully covered the Petri plates in any one of the treatments.

Effect of aqueous extracts of leaves of bhendi varieties on conidial germination of *B. bassiana* was assayed by cavity slide method. Leaf extracts and 0.05 ml of spore suspension of *B. bassiana* were mixed in a cavity slide and incubated for 24 hrs. Cavity slides with sterilized distilled water were treated as control. Observations were taken from 20 microscopic fields for each slide and total number of conidia germinated in each microscopic field

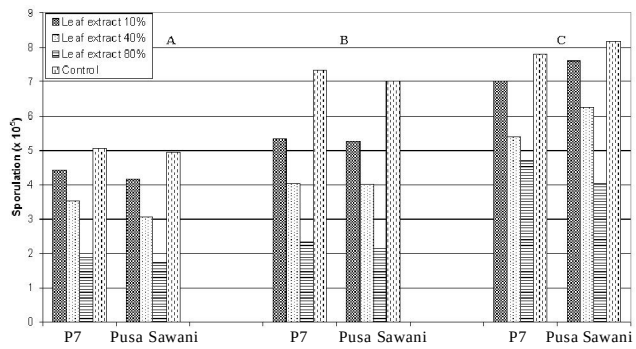

Figure 1. Effect of leaf extracts (in %) of bhendi varieties of sporulation of *B.bassiana* of 0.1 % (A), 1. 07. (B) and 3.0% (C) concentrations

was estimated using a haemocytometer and per cent germination was calculated. Similarly, a single mycelial disc from the growing point was removed with the aid of sterile cork borer and transferred into one ml sterile distilled water and shaken vigorously. A drop of spore suspension was placed on a glass slide and number of spores present in each microscopic field was recorded.

Five replications were maintained per treatment in the above experiments and the data gathered were analysed as per the methods described by Gomez and Gomez (1983) using computer based IRRISTAT analysis developed by International Rice Research Institute, Philippines. The percentage data were transferred to arc sine values before subjecting to analysis of variance.

Table 2. Effect of leaf extracts of bhendi varieties on mycelial growth of *B. bassiana* at various concentrations

Bhendi varieties	Mycelial growth (mm) of *B. bassiana*				
	Concentration of leaf extracts (%)				
	10	40	80	Control	Mean
	0.1%				
P 7	41.58	30.40	22.60	48.10	35.67
Pusa Sawani	37.70	29.32	18.00	40.38	31.35
Mean	39.64	29.86	20.30	44.24	
	1%				
P 7	54.12	45.94	30.16	58.32	47.13
Pusa Sawani	49.60	42.60	27.98	54.20	43.59
Mean	51.86	44.27	29.07	56.26	
	3%				
P 7	64.08	56.56	36.48	64.30	55.35
Pusa Sawani	58.08	41.48	29.44	60.32	47.33
Mean	61.08	49.02	32.96	62.31	
C.D.	Between Varieties	Among Trt.	Varieties x Trt.		
0.1%	0.85	1.21	1.71		
1%	0.69	0.98	1.39		
3%	1.75	2.48	3.51		

Each value is a mean of five replications

RESULTS AND DISCUSSION

Though earlier works on EPF were focused mainly on evaluating the bioefficacy under laboratory or field conditions, very limited works have been done on ascertaining the role of host plant factors in influencing the pathogenicity of EPF. Hence the present investigation was carried out to study the tri-trophic interaction of host plant factors with *B. bassiana* infecting *S. litura* in bhendi. In the laboratory bioassay, the mortality of *S. litura* larvae differed significantly between the bhendi varieties both in the topical and contaminated food bioassay at various concentrations (Table 1). Higher larval mortality was observed in topical bioassay than contaminated food bioassay. Irrespective of the bhendi variety, mortality of *S. litura* larvae increased with the increase in the dose of *B. bassiana*. In the topical bioassay, significantly high larval mortality was recorded in the tolerant variety P 7 as against the susceptible variety Pusa Sawani. But in the contaminated bioassay, both the varieties were statistically on par with each other at 0.1 and 1 per cent concentration of *B. bassiana* (Table 1).

When the leaf extracts of the two bhendi varieties were amended to the culture medium, the least mycelial growth and conidial germination was recorded at 80 per cent concentration in case of both the varieties (Table 2, Fig 1). The control treatment recorded the maximum mycelial growth and conidial germination followed by 10 per cent leaf extract of both varieties. The minimum mycelial growth and conidial germination at the higher concentrations of the leaf extracts may be due to the reduction of carbon source in the culture medium. Further, at higher concentrations, the secondary metabolites in the leaves may also inhibit the growth of *B. bassiana*. Similarly, alkaloids such as tomatine and solanine are reported to inhibit EPF (Costa and Gaugler, 1989b; Gallardo *et al.*, 1990; Lacey and Mercadier, 1998) and affect conidial germination (Poprawski *et al.*, 2000). Between the two bhendi varieties, the maximum mycelial growth and conidial germination was recorded in the variety P 7. *B. bassiana* at 3.0 per cent concentration recorded the maximum conidial germination since higher the inoculum load, higher would have been the subsequent conidial germination.

The sporulation rate was higher at higher concentration of *B. bassiana* but it was reduced considerably at higher doses of leaf extracts of both the varieties (Fig 1). Hence, it may be concluded that the host plant factors though exert a significant influence on the growth of entomopathogenic fungi, its influence on infectivity at the field level depends on the nature, concentration and availability of secondary metabolites.

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T. Suganya and V. Selvanarayanan*
Department of Entomology, Faculty of Agriculture, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India, Phone: 9443332024 *Email: selvaento @gmail.com.