Journal of Biopesticides 3(1 Special Issue) 143 - 146 (2010) 143

Effect of commonly used insecticides on the growth of white Muscardine fungus, *Beauveria bassiana* under laboratory conditions

M. Amutha, J. Gulsar Banu, T. Surulivelu and N. Gopalakrishnan

ABSTRACT

Compatibility of *Beauveria bassiana* was studied in the laboratory condition with twelve commonly used insecticides for cotton pest management by poisoned food technique. The results were expressed as percentage of growth inhibition of *B. bassiana* colony on insecticide treated medium. Among the insecticides tested for their compatibility, only chlorpyriphos 20 EC was rated as relatively less toxic to *B. bassiana,* while, spinosad (45 % SC), econeem (1%), quinalphos (25 EC), acetamprid (20%), endosulfan (35 EC) and thiodicarb (75 WP) were slightly toxic. Imidacloprid (17.80% SL) and triazophos (40 EC) were moderately toxic and profenofos (50 EC), indoxacarb (14.5 % EC) and methyldemeton were highly toxic. Results of the present study suggested that except profenophos, indoxacarb and methyldemeton, the rest of the insecticides tested can be safely used along with the entomopathogenic fungi *B. bassiana*.

Key words: compatability, *Beauveria bassiana,* insecticide, cotton

INTRODUCTION

Entomopathogenic fungi are important natural biocontrol agents of many insect pests. Biological control, in particular when accomplished by entomopathogens, is a technique that should be considered as an important pest population density reduction factor in Integrated Pest Management (IPM) programs. *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) is a registered biopesticide with a broad host range of approximately 700 insect species used for management of several crop insect pests. The integration of microbial pesticides with chemical pest management practices requires detailed compatibility studies. Data from such studies would enable farmers to select appropriate compounds and schedule microbial and chemical pesticide treatments so that benefits from compatible sets can be accrued and, with noncompatible pairs, the deleterious effect of the chemical on the microbe in the biopesticide can be minimized (Butt *et al*., 2001; Inglis *et al*., 2001; Lacey *et al*., 2001). A microbial pesticide compatible with a commonly used chemical pesticide can be used simultaneously or sequentially with it. To harness the benefits of entomopathogenic fungus their compatibility with insecticides becomes decisive for combined use, while the potential inhibitory effects of insecticides on the entomopathogenic fungus cannot be ignored. The use of incompatible insecticides may inhibit the development and reproduction of these pathogens, affecting IPM. IPM programmes it is essential to know the influence of compatibility between entomopathogenic fungi and pesticides used in crop protection. If *B. bassiana* has to be incorporated into a pest management programme it is essential to determine the effects of pesticides on it. Therefore keeping this in view, present investigations were taken-up to study the compatibility of insecticides with *B. bassiana* at *in-vitro* condition.

MATERIALS AND METHODS

The present study was conducted at Central Institute for Cotton Research, Regional station, Coimbatore, Tamil Nadu, India. The experiment was laid out by completely randomized block design. The insecticides selected for compatibility study were among those commonly used for pest management. The effect of these insecticides on the radial growth and germination of *B. bassiana* was evaluated. The insecticides dose was calculated for field application rate based on 500 litres of spray fluid/ha. Twelve insecticides were evaluated by poisoned food technique (Moorhouse *et al*., 1992) in Potato Dextrose Agar (PDA) medium. Twenty ml of PDA medium was sterilized in individual boiling tubes and the insecticide emulsions of required concentration were incorporated into the melted sterile PDA aseptically, thoroughly mixed, poured into 9 cm diameter sterile Petri dishes and allowed to solidify under laminar flow cabinet. An agar disc along with mycelium mat of *B. bassiana* was cored from the periphery of 10 day old colony of *B. bassiana* by 10mm diameter cork borer and transferred in to the centre of the PDA plate. Growth medium (PDA) without insecticide but inoculated with mycelial disc served as untreated check. The plates were sealed with parafilm and incubated at room temperature for 14 days to allow maximum growth. Each treatment was replicated three times. The diameter of growing culture in excess of the plugs in each Petri dish was measured on 14 days after inoculation (DAI) (when radial growth in the control plate fully covered the medium) and also on 30 days after inoculation. The data were expressed as percentage growth inhibition of *B. bassiana* by insecticide treated PDA (Hokkanen and Kotiluoto, 1992).

$$X = \frac{Y-Z}{Y} \times 100$$

where X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in untreated check and radial growth of fungus in poisoned medium, respectively. The pesticides were further classified in evaluation categories of 1- 4 scoring index. 1 = harmless (<50% reduction in beneficial capacity), 2 = slightly harmful (50-79%), 3 = moderately harmful (80-90%), 4 = harmful (>90%) in toxicity tests *in vitro* according to Hassan's classification scheme (Hassan, 1989).

The data obtained from various experiments were statistically analysed and subjected to Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) using IRRISTAT version 92- a developed by International Rice Research Institute, Biometrics Unit, Philippines.

RESULTS AND DISCUSSION

The effects of insecticides on the mycelial growth of *B. bassiana* are shown in Table 1. All the treatments showed significant differences relating to control. Among the insecticides tested, only chlorpyriphos was relatively less toxic to *B. bassiana* (Hazardless). Six insecticides (spinosad, econeem, quinalphos, acetamprid, endosulfan and thiodicarb) proved relatively slightly toxic, two insecticides (imidacloprid and triazophos) were moderately toxic and three insecticides (profenofos, indoxacarb and methyldemeton) were highly toxic, among these, indoxacarb induced high reduction in mycelial growth of *B. bassiana*. On thirty days after inoculation, only chlorpyriphos was rated as hazardless, eight insecticides (spinosad, econeem, quinalphos, acetamprid, endosulfan, imidacloprid, triazophos and thiodicarb) were rated as slightly toxic, one insecticide (profenophos) was rated as moderately toxic and two insecticides (indoxacarb and methyldemeton) were rated as highly toxic. Compatibility of chemical pesticides with the mycopathogen have been studied by Fargues (1975), Anderson *et al.* (1989), Mohammed *et al.* (1987) and Castinerias *et al.* (1991). Hassan and Charnely (1989) revealed the in-consistent interaction between fungus and insecticides. In our present study chlorpyriphos showed no toxic effect to *B. bassiana*. In earlier reports, chlorpyriphos and monocrotophos were found to be slightly harmful to *B. bassiana* at normal field dose (Ambethgar, 2003). Chlorpyriphos had been reported to strongly inhibit the growth and sporulation of *B. bassiana* (Barbosa and Moreira, 1982) in a dose-dependent manner even at concentrations lower than recommended rates of field use (Rao, 1989). Masarat (2009) reported that chlorpyriphos and endosulfan strongly inhibit the growth of *B. bassiana.* Oliviera *et al.* (2003) reported triazophos, chlorpyriphos and endosulfan formulations inhibited 100% of the germination of *B. bassiana.* According to James and Elzen (2001) and Alizadeh *et al.* (2007), imidacloprid had no negative effect on *B. bassiana.*

With regard to the neem based insecticides tested in the present study, azadirachtin 3 per cent (Econeem) was found to be slightly harmful. Jayaraj (1988) hinted the possibility of combining botanicals with microbial for enhanced efficacy against insect pests. The compatibility of isolates of *B. bassiana* with azadirachtin formulations has been investigated previously (Rodrýguez *et al.*, 1997; Bajan *et al.*, 1998; Gupta *et al.*, 1999; Depieri *et al.*, 2005). However, a few isolates were tested and contradictory results have been reported. For example, neem oil was found compatible with *B. bassiana* by Rodrýguez *et al.* (1997) and Ambethgar, 2003, but was reported to be inhibitory by Bajan *et al.* (1998) and Depieri *et al.* (2005). The observed difference could be due to inherent variability of chemicals to biological creatures.

Results of the present study thus reveal that, among the selected insecticides, except a few (profenofos, indoxacarb, imidacloprid, triazophos and methyldemeton) all other insecticides can be safely used along with the mycopathogen. While comparing toxicity of insecticides against *B. bassiana* on 14 days and 30 days after inoculation, toxicity of insecticides was reduced on 30 days after inoculation. On 14 DAI, imidacloprid and triazophos was moderately toxic, during 30 DAI its toxicity reduced to slightly toxic. Highly toxic insecticide profenophos on 14 DAI was degraded to moderately toxic on 30 DAI.

Although the different insecticides tested in the present investigations inhibited the growth of *B. bassiana* in the poisoned medium *in vitro*, the combined use of the fungus and insecticides cannot be completely ruled out. Certain

Table 1. Effect of insecticides on the growth of *Beauveria bassiana*

Treatments	14 days after inoculation			30 days after inoculation		
	Mean diameter of mycelial * growth (mm)	Per cent reduction over control	Grade	Mean diameter of mycelial growth (mm)	Per cent reduction over control	Grade
Chlorpyriphos 20 EC	34.67[b]	49.76	1	39.17[b]	48.46	1
Spinosad 45 % SC	14.67[h]	78.74	2	20.00[h]	73.68	2
Profenofos 50 EC	4.33[k]	93.72	4	12.50[k]	83.55	3
Imidacloprid 17.80% SL	13.67[i]	80.19	3	16.00[j]	78.95	2
Triazophos 40 EC	10.33[j]	85.02	3	27.67d	63.60	2
Econeem 1%	30.33[c]	56.04	2	32.33[c]	57.46	2
Quinalphos 25 EC	21.33[f]	69.08	2	24.50[g]	67.76	2
Acetamprid 20%	22.33[e]	67.63	2	25.33[f]	66.67	2
Indoxacarb 14.5 % EC	0.00[m]	100.00	4	0.00[m]	100.00	4
Endosulfan 35 EC	16.67[g]	75.85	2	19.00[i]	75.00	2
Thiodicarb 75 WP	25.67[d]	62.80	2	27.33[e]	64.04	2
Methyldemeton	3.33[l]	95.17	4	4.67[l]	93.86	4
Untreated check	69.00[a]	-	-	76.17[a]	-	-

In a column, mean followed by common letters are not significantly different at five per cent level (DMRT)

insecticides have been combined at sub-lethal doses with entomopathogenic fungi for obtaining better control of the pest species. For instance, increased mortality due to mycosis of *Beauveria* by addition of reduced doses of insecticides had been established in *Melolontha melolontha* L. (Ferron, 1971). Similarly, Benz (1971) established positive results on the combined use of reduced doses of insecticides with *B. bassiana* for the combined use of reduced doses of insecticides with *B. bassiana* for the control of coleopterous insects. Considering this, it is worth exploring the effects of these insecticides at sub-lethal doses on the fungus as two-in-one mix strategy. While doing so, adequate pre caution should also be taken because these compounds at sub-lethal doses may end up with complications like resurgence of sucking insects.

The laboratory results on artificial media may not be reproducible in field as there will be degradation of toxicants. Thus, when the product is determined in the laboratory, no doubt that its selectivity under filed condition will stand. On the other hand, the high toxicity under *in vitro* of a given formulation may suggest similar toxicity under field conditions. However for field studies the inhibition of conidial germination should be the key factor to be considered. It needs field confirmation. The mycopathogen, to be considered as an integral part of plant protection has to meet the requirement of compatibility with many other measures recommended for management of pests. Interval between insecticide spray and the mycopathogen inoculation decides the effectiveness and strategy development for the conjunctive or supplementary use of mycopathogens. New techniques through biotechnology or genetic engineering may be helpful for strain improvement of fungal pathogens for tolerance to agrochemicals.

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M. Amutha*, J. Gulsar Banu, T. Surulivelu and N. Gopalakrishnan

Central Institute for Cotton Research, Regional Station, Coimbatore-3, Tamil Nadu, India, Phone: 099427 92667, Fax: 0422-245021, *E-mail: amuento @yahoo.co.in