

Original Research Article

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Compatibility of Entomopathogenous Fungi with Commonly used Insecticides for Management of Banana Aphid transmitting *Banana bunchy Top virus* (BBTV) in Assam Banana Production System

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ABSTRACT

Keywords

Entomopathogenic fungi, *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii*, Pesticides

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The three entomopathogenic fungi viz., *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Verticillium lecanii* were tested for their compatibility with three insecticides used in banana production system in *in vitro* by poison food technique. All the insecticides were tested for their compatibility with each fungi at three different concentrations i.e., recommended dose (RD), half of the RD and one fourth of the RD. *V. lecanii* was found highly compatible with all the insecticides followed by *B. bassiana*. The concentration ¼ RD of all the insecticides was found safe showing higher mycelial growth of the fungi. Imidacloprid 17.8% SL at 0.025 % showed no inhibition (0.00 %) of *V. lecanii* followed by 9.81 per cent to *B. bassiana*. Dimethoate 30 EC at 0.05% showed maximum inhibition of 21.25 per cent of *V. lecanii* followed by 27.81 per cent to *B. bassiana*. The botanical insecticide Azadirachtin at 0.075% showed maximum inhibition of 22.94 per cent of *V. lecanii* followed by 35.19 per cent to *B. bassiana*. Present investigations showed effects of pesticides on the fungi, their actual effects at cellular and field level need to be investigated for the efficient utilization of entomopathogens in the integrated pest management of banana.

Introduction

To control the secondary infection of *Banana bunchy Top virus* (BBTV) transmitted by its insect vector *Pentalonia nigronervosa* in banana field there is a need to find out alternative agents that are pest specific, non-toxic, biodegradable, safe to natural enemies, less prone to resistance and less expensive. In the integrated pest management (IPM) schedule against the insect-vector, inclusion of neem based formulations as well as biocontrol agents (BCAs) like entomopathogenic fungi

has been adopted globally (Alves and Lecuona, 1998; Ramarethinam *et al.*, 2004). A lot of examples exist where application of different selective chemical insecticides and fungi when used in combination provide satisfactory control against many agricultural insect pests (Serebrove *et al.*, 2005; Purwar and Sachin, 2006). On the other hand, the use of non selective or incompatible chemical pesticides may possibly have the potential to hinder the vegetative growth and development of fungi adversely affecting the IPM (Duarte *et al.*, 1992 and Malo, 1993). For this reason,

an understanding about the adverse effects of different insecticides on entomopathogenic fungi is necessary. A number of experiments have been done to evaluate the deleterious effects of chemical insecticides on different developmental stages of fungi (Aliazadeh *et al.*, 2007). The effect of these products may vary in different species and strains of fungi (Anderson *et al.*, 1989). The results from such experimental work would direct the farmers to choose a more compatible pesticides and the adverse effects of the injudicious use of insecticides can be minimized (Butt *et al.*, 2001 and Inglis *et al.*, 2001). Therefore there is a need to manipulate the inhibitory effects of different insecticides on the mycelial growth and sporulation of isolates of different BCAs, as well as, to check the compatibility of these chemicals with these BCAs.

Materials and Methods

The three entomopathogenic fungi *viz.*, *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metchnikoff) Sorokin and *Verticillium lecanii* used in this study were collected from culture collection of Mycology Research Section, Department of Plant Pathology, Faculty of Agriculture, AAU, Jorhat. All the three organisms were cultured on PDA medium in 8 cm petri dishes and incubated at 25 ± 2 °C temperature. Three different pesticides *viz.*, Azadirachtin (0.15%) @ 3ml/l (Margosom), Imidacloprid 17.8% SL @ 1ml/l (Imidacel) and Dimethoate 30 EC (0.06%) @ 2ml/l (Rogor) used in banana production system were selected to assess the compatibility with BCAs. For compatibility tests the pesticides were used in three different concentrations, *i.e.*, recommended dose (RD) (lethal), half of the recommended dose ($\frac{1}{2}$ RD) (sub lethal) and one fourth of the recommended dose ($\frac{1}{4}$ RD) (sub-sub lethal).

Standard poisoned food technique was followed to assay the *in vitro* compatibility of

entomopathogens with the different pesticides (Nene and Thapliyal, 1997) (Plate 1). Quantity of pesticides used was based on field application rate with high volume sprayer. Standard PDA medium (30 ml) was autoclaved at 121°C for 15 minutes, cooled to 40 ± 5 °C, and amended with 0.3 g/l of streptomycin sulphate. The required concentrations (RD, $\frac{1}{2}$ RD and $\frac{1}{4}$ RD) of pesticides (30ml each) were prepared and added to the media while it was warm and agitated thoroughly to get a uniform distribution of pesticides in the media. For control plates, appropriate amount of streptomycin sulphate (0.3g/l) alone was added.

Approximately 15 ml of each of the amended media was poured aseptically into 8 cm sterilized petri dishes. The same amount of medium without the pesticides was used as control for comparison under the same conditions. Mycelial disc (5mm diameter) of young fungal mycelium was cut with sterile cork borer and placed aseptically in the centre of each petri dish containing the poisoned medium. Inoculated petri dishes were incubated at 27 ± 1 °C, and 80 ± 5 per cent relative humidity. For each treatment four replications were maintained. Suitable check without poison was kept for comparison under the same condition. Fungal colony diameter was measured with a caliper ruler after every 24 hrs of inoculation till full growth was observed in control plates. Percent inhibition of radial growth of fungal isolate over untreated check was worked out for the respective chemicals by following the formula:

Per cent inhibition of radial growth =

$$\frac{(C - T)}{C} \times 100$$

Where, C = colony diameter of BCA in control

T = colony diameter of BCA in treatments

Further, the data were statistically analysed and critical difference (CD) calculated at P = 0.05. The experiment was laid out in Completely Randomized Block Design (CRD) with 30 treatments in 4 replications. Fisher's Method of analysis of variance was followed for statistical analysis of the experimental data. Significance of variance among data were calculated out by calculating the 't' at 5 per cent probability level. Different treatment means were compared with critical difference (C.D.). Means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

In vitro, evaluation of compatibility of different biocontrol agents (BCAs) with botanical and chemical pesticides showed that *V. lecanii* was highly compatible with all the pesticides followed by *B. bassiana* and *M. anisopliae* (Table 1). The maximum growth of the BCAs were recorded at ¼ RD (sub-sub lethal dose) followed by ½ RD (sub lethal) and RD (lethal) of the three pesticides. Among the pesticides tested, Imidacloprid at 0.025% (¼ RD) showed maximum radial growth of 80.00 mm in the treatment combination of Imidacloprid @ 0.025% + *V. lecanii* with no inhibition (0.0%) on growth of the fungus followed by 72.15 mm in Imidacloprid @ 0.025% + *B. bassiana* and 55.35 mm in Imidacloprid @ 0.025% + *M. anisopliae*. The per cent inhibition of radial growth of *B. bassiana* and *M. anisopliae* over control was found to be 9.81 and 11.51 per cent, respectively. Dimethoate at 0.05% (¼ RD) showed highest radial growth of 63.00 mm with growth inhibition of 21.25 per cent in combination of *V. lecanii* followed by *B. bassiana* with 57.75 mm growth and 27.81 per cent growth inhibition. This was followed by *M. anisopliae* with 24.35 mm growth and 61.07 per cent inhibition. The maximum radial

growth or colony diameter of the three BCAs with Azadirachtin was recorded at the one fourth concentration of the recommended dose (¼ RD) *i.e.*, 0.075 per cent. Azadirachtin @ 0.075 % + *V. lecanii* showed 61.65 mm growth with 22.94 per cent inhibition followed by Azadirachtin @ 0.075 % + *B. bassiana* (51.85 mm and 35.19 per cent) and Azadirachtin @ 0.075% + *M. anisopliae* (25.90 mm and 58.59 per cent), respectively.

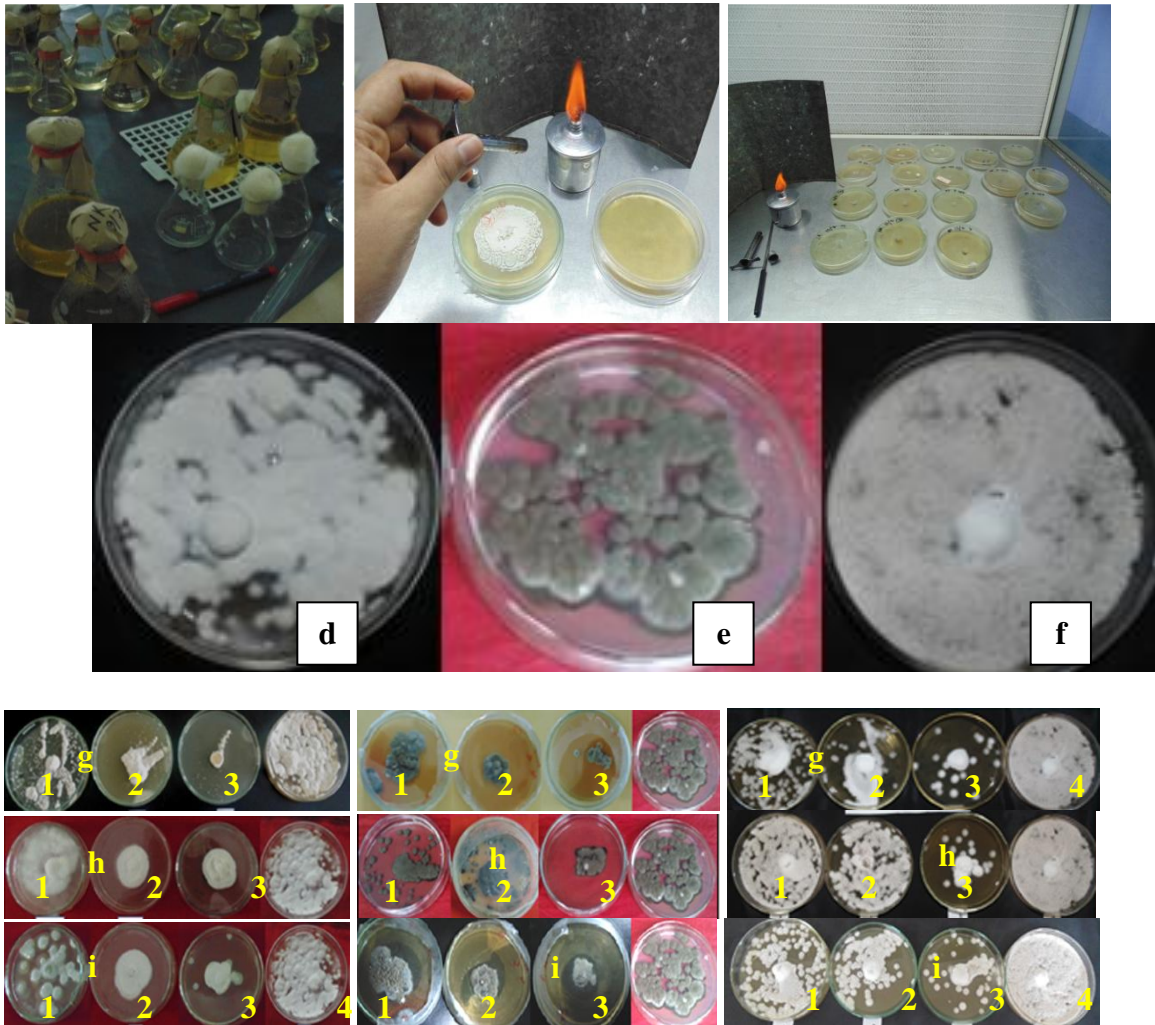
The highly compatible nature of *V. lecanii* with different pesticides were observed by Li *et al.*, (2002), Derakhsan (2006), Manjunatha *et al.*, (2006), Madhavji (2007) and Alizadeh *et al.*, (2007) who reported the least inhibition of mycelial growth of *V. lecanii* with Imidachloprid, neem seed kernel extract(NSKE) and Dimethoate. They also reported that these chemicals were less toxic even at their recommended dose of field application. Sharma and Gupta (1998) reported that Azadirachtin (0.03% EC), compatible with *B. bassiana* even upto 2000 ppm. The results of evaluation of compatibility of *B. bassiana* were in accordance with that of Azadirachtin and Dimethoate as reported by Puzari *et al.*, (2006). Similar results were observed when compatibility of *B. bassiana* was evaluated with Imidachloprid by Alizadeh *et al.*, (2007). The fungal biocontrol agents and pesticides may act synergistically increasing the efficiency of the control, allowing lower doses of pesticides (Moino and Alves, 1998). Further, compatible combination can reduce the cost of cultivation by reducing the time of application of different component singly. The observed variations in the inhibitory potential could be due to inherent variability of chemical insecticide to biocontrol agents. Their inhibitory potential varies both between and within chemical classes (Inglis *et al.*, 2001). A given insecticides may have different fungitoxic effects on various developmental stages of the fungus (Li and Holdom, 1994).

Table.1 *In vitro* compatibility of Biocontrol agents (BCAs) with botanical and chemical pesticides

Treatment combinations (Pesticides+ BCA)	Radial growth of BCA (mm)*	Per cent Inhibition of radial growth over control
Azadirachtin (0.15%) @ 0.075 % + <i>B. bassiana</i>	51.85 ^{efg}	35.19
Azadirachtin (0.15%) @ 0.15 % + <i>B. bassiana</i>	36.90 ^h	53.88
Azadirachtin (0.15%) @ 0.3 % + <i>B. bassiana</i>	19.75 ^{klmn}	75.31
Azadirachtin (0.15%) @ 0.075 % + <i>M. anisopliae</i>	25.90 ^{kl}	58.59
Azadirachtin (0.15%) @ 0.15 % + <i>M. anisopliae</i>	17.40 ^{mn}	72.18
Azadirachtin (0.15%) @ 0.3 % + <i>M. anisopliae</i>	15.40 ⁿ	75.38
Azadirachtin (0.15%) @ 0.075 % + <i>V. lecanii</i>	61.65 ^d	22.94
Azadirachtin (0.15%) @ 0.15 % + <i>V. lecanii</i>	51.00 ^{fg}	36.25
Azadirachtin (0.15%) @ 0.3 % + <i>V. lecanii</i>	45.60 ^g	43.00
Imidacloprid 17.8% SL @ 0.025 % + <i>B. bassiana</i>	72.15 ^b	9.81
Imidacloprid 17.8% SL @ 0.05 % + <i>B. bassiana</i>	34.50 ^{hi}	56.88
Imidacloprid 17.8% SL @ 0.1 % + <i>B. bassiana</i>	25.70 ^{kl}	67.88
Imidacloprid 17.8% SL @ 0.025 % + <i>M. anisopliae</i>	55.35 ^{def}	11.51
Imidacloprid 17.8% SL @ 0.05 % + <i>M. anisopliae</i>	46.40 ^g	25.81
Imidacloprid 17.8% SL @ 0.1 % + <i>M. anisopliae</i>	26.15 ^{kl}	58.19
Imidacloprid 17.8% SL @ 0.025 % + <i>V. lecanii</i>	80.00 ^a	0.00
Imidacloprid 17.8% SL @ 0.05 % + <i>V. lecanii</i>	69.20 ^{bc}	13.50
Imidacloprid 17.8% SL @ 0.1 % + <i>V. lecanii</i>	30.65 ^{hij}	61.69
Dimethoate 30EC (0.06%) @ 0.05% + <i>B. bassiana</i>	57.75 ^{def}	27.81
Dimethoate 30EC (0.06%) @ 0.1% + <i>B. bassiana</i>	37.10 ^h	53.63
Dimethoate 30EC (0.06%) @ 0.2% + <i>B. bassiana</i>	31.20 ^{hij}	61.00
Dimethoate 30EC (0.06%) @ 0.05% + <i>M. anisopliae</i>	24.35 ^{klm}	61.07
Dimethoate 30EC (0.06%) @ 0.1%+ <i>M. anisopliae</i>	27.25 ^{ijk}	56.43
Dimethoate 30EC (0.06%) @ 0.2% + <i>M. anisopliae</i>	19.25 ^{lmn}	69.22
Dimethoate 30EC (0.06%) @ 0.05% + <i>V. lecanii</i>	63.00 ^{cd}	21.25
Dimethoate 30EC (0.06%) @ 0.1% + <i>V. lecanii</i>	58.85 ^{de}	26.44
Dimethoate 30EC (0.06%) @ 0.2% + <i>V. lecanii</i>	30.00 ^{hij}	62.50
<i>B. bassiana</i> alone (control)	80.00 ^a	
<i>M. anisopliae</i> alone (control)	62.55 ^{cd}	
<i>V. lecanii</i> alone (control)	80.00 ^a	
SEd	0.35	
CD(P=0.05)	0.69	
CV	10.96	

*Data are mean of four replications
 Mean followed same letter are at par with each other

Plate.1(a-i) A general view of *in vitro* compatibility test of entomopathogenic fungi with pesticides
d. *Beauveria bassiana*
e. *Metarhizium anisopliae*
f. *Verticillium lecanii*
g. Azadirachtin (0.15%)
h. Imidacloprid (17.8% SL)
i. Dimethoate (30EC, 0.06%)
(Where, 1: One fourth of recommended dose (RD), 2: Half of RD, 3: RD and 4: Control)



The potential inhibitory effects of pesticides on germination and mycelia growth of biocontrol fungi vary from taxa and strains (Anderson *et al.*, 1989). However results may differ in field because fungi are exposed maximum to pesticides *in vitro* which doesn't occur under field conditions. Additionally,

fungi may recover after some chemical pesticides are decomposed on plant leaves. Therefore, once a chemical insecticide is proved to be compatible in the laboratory, it must be selective in field conditions. On the other hand, high *in vitro* toxicity of the product will not always be same in the field

(Butt and Brownbridge, 1997) but is likely to be occur (Alves *et al.*, 1998). Present investigations showed varying effects of pesticides on the fungi, their actual effects at cellular and field level need to be investigated to understand if the effects are permanent or temporary. In case of temporary arrest of fungus activity, it may recover after degradation of toxicant and such chemicals can be employed in combination with the fungus under field conditions. The results for Compatibility analysis of biocontrol agents with chemical and botanical pesticides used in banana production system are of permanent importance in developing strategies for the efficient utilization of entomopathogens in the integrated pest management of banana.

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