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Full Length Research Paper

Compatibility of entomopathogenic fungi with extracts of plants and commercial botanicals

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The compatibility of some commercial botanicals (Biospark, Phytophrate, Exodos, Biodos and Neemgold) and of solvent extracts of *Syndrella nodiflora*, *Premna tomentosa*, *Vitex negundo*, *Ipomea carnea*, *Pteridium aquilinum* (leaves) and *Annona squomosa* (seeds) with *Beauveria bassiana* (Bals.) Vuil., *Isaria (Paecilomyces) fumosorosea* (Wize) Brown et Smith and *Lecanicillium* (*Verticillium) lecanii* Humber *in vitro* was studied using dual plate and liquid bioassays. The results showed that Biospark. Phytophrate and Exodos highly reduced the mycelial growth of *B. bassiana, P. fumosorosea* and *V. lecanii*, respectively. Irrespective of the fungi tested, *A. squomosa* ethanol and *I. cornea* water extracts had maximum and minimum growth inhibiting activity against three fungi, respectively. Hence, these extracts can be integrated along with these fungi in the biointensive integrated pest management (BIPM) programme.

Key words: Compatibility, entomopathogenic fungi, plant-based biopesticides, plant extracts.

INTRODUCTION

Investigations are going on throughout the world to discover safer and effective methods to control insect pests. Entomopathogenic bacteria, viruses, fungi and nematodes have been reported as plant protection agents against several insects (Rosell et al., 2008). Biological control, particularly by entomopathogenic fungi, is important for reducing the population density of pests in Integrated Pest Management (IPM) programs. The entomopathogenic fungi, *Beauveria bassiana* (Balsamo), *Vaillemin* (Todorova et al., 1996; Padmaja, 1998; Ying et al., 2003; Ming-Guang, 2004; Ajaykumar, 2008), *Isaria fumosorosea* (Wize) and *Lecanicillium lecanii* (Zimm.) Viegas (Milner and Lutton, 1986; Dekrakhsgan et al., 2007; Vasantharaj, 2008; Sahayaraj and Namasivayam, 2008) are the most promising biological control agents and their role in the regulation of insect populations is well documented. The need to preserve entomopathogens that occur naturally, or are introduced for insect control (Oliveira et al., 2003) necessitates a proper understanding of the compatibility of entomopathogenic fungi with other crop protection techniques such as the use of insecticides, which may inhibit to a smaller or larger extent the development and reproduction of pathogen (Malo, 1993).

Insecticidal properties of plants such as *Syndrella nodiflora* Gaertn (Rathi and Gopalakrishnan, 2005), *Premna tomentosa* Willd. (Rathi and Gopalakrishnan, 2006), *Vitex negundo* (Sahayaraj, 1998; Sahayaraj et al., 2007a), *Ipomea carnea* (Sahayaraj et al., 2003), and *Pteridium aquilinum* (Sahayaraj et al., 2007b) and seeds of *Annona squomosa* Linn. (Raman et al., 2007) have been reported earlier. Increasing awareness about the use of entomopathogenic microorganisms and several plant-based have been recommended in IPM programme. A combination of entomopathogenic fungi with plant-based insecticides may provide a more sustainable pest management strategy at reduced cost. It is therefore, necessary to determine the compatibility of botanicals

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Abbreviations: BIPM, Biointensive integrated pest management; **IPM,** integrated pest management; **PDA,** potato dextrose agar; **NSKE,** neem seed kernel extract; **SMYA,** sabourad maltose agar enriched with 1% yeast extract.

with entomopathogenic fungi of present and future importance to maximize their combined efficacy. The present study was undertaken to evaluate the impact of three fungal pathogens viz *B. bassiana, P. fumosorosea* and *V. lecanii* on some selected plant-based commercially available biopesticides (Biospark, Phytophrate, Exodos, Biodos and Neemgold) and some plant extracts (aerial parts of *S. nodiflora*, *P. tomentosa*, *V. negundo*, *I. carnea*, *P. aquilinum* and seed of *A. squomosa*), which possess insecticidal properties against insect pests of the groundnut ecosystem.

MATERIALS AND METHODS

Entomopathogenic fungal culture

Fungi were isolated from the diseased caterpillars of *Spodoptera litura* collected from the groundnut fields in Tamil Nadu, India. The diseased larvae showed white color for *B. bassiana* and slight reddish mycelial surface growth for *I. fumosoroseus*. The diseased larvae were collected in screw cap vials (18 x 4 mm) and brought to the laboratory for further studies. The diseased larvae were surface sterilized with 0.1% mercuric chloride for few seconds and then thoroughly washed with sterilized double distilled water. The excess water was removed by keeping the diseased larvae on Whatman No. 1 filter paper. The diseased larvae were cut into small pieces with a sterile blade and the pieces aseptically transferred on to the Sabourad maltose agar enriched with 1% yeast extract (SMYA) slant using sterile inoculation needle. The slants were kept at 25 ± 1°C. Diseased larvae were also kept on moist filter paper in Petri dish for mycelial growth and sporulation. The fungi were identified based on the morphological characters as per Humber (1997). The standard fungi *B. bassiana, I. fumosorosea* and *L. lecanii* were obtained from Microbial Type Culture Collection (MTCC 915), Chandigarh, India. All the cultures were maintained on SMYA and potato dextrose agar (PDA) slants. In order to maintain the virulence of the fungus, they were re-isolated from *S. litura* infected with conidial application in the laboratory as per Koch postulates.

Commercial plant-based pesticides

Commercially available plant-based products such as biospark (Fastura Lab Science (P) Ltd., Hydrabad) (0.5%), phytophrate (0.5%) (Elibec Inoculate Ltd., Bangalore), exodos and biodos (0.5%) (Jasmine Biological (P) Ltd., Hydrabad) and neemgold (0.5%) (Shri Disha Biotech. Ltd., Hydrabad) and the fungicide carbendazim (0.01%) were obtained from the respective companies and used for the present study.

Crude plant extracts preparation

Aerial part of *S. nodiflora*, *P. tomentosa*, *V. negundo*, *I. carnea* and *P. aquilinum* and seeds of *A. squomosa* were collected from Tirunelveli District, Tamil Nadu, India. The plant materials were washed thrice with tap water, once with distilled water and then shade dried for two weeks. Dry aerial part and seeds were stored at ambient temperature indoor until needed. For extraction, 200 g of the aerial part and seed separately were ground using a household blender and stored in a refrigerator for further use. From the stock, 150 g of powder was extracted twice with 300 ml of respective solvents (*S. nodiflora* - benzene and methanol, *V. negundo*-methanol and water, *A. squomosa* - ethanol and water, *I. carnea* - methanol and water, *P. tomentosa* - benzene and ethanol and *P. aquililum*-Hexane) (Nice, Cochin) subsequently using soxhlet apparatus for about 24 h separately. The solvents were removed using a distillation apparatus and dark green color extract from aerial parts and dark brown colored extract from seed were obtained and refrigerated for up to 4 weeks before use. These crude extracts were used for preparing stock solution. The known amount of (100 mg/ml) of crude extract obtained from the above process was diluted to obtain the desired concentration (3%). A drop of emulsifier Tween 80 (Himedia, Mumbai) was added with the respective solvent extracts to ensure complete solubility of the material in water.

Dual plate assay

Preliminary *in vitro* studies were undertaken in the laboratory to assess the compatibility of five plant based pesticides and the common fungicide carbendazim as a standard check with *B. bassiana, I. fumosorosea* and *L. lecanii* by adopting poisoned food technique (Olmert and Kenneth, 1974). Recommended field concentration of the plant products (0.5%) and carbendazim (0.01%) were added to the sterilized SMYA and poured to the Petri plate after proper agitation and allowed to solidify. Fungal disc from fully-grown (15 days old culture) *B. bassiana, I. fumosorosea* and *L. lecanii* culture plate was transferred from the culture plate with the help of a sterilized cork borer of 8 mm size to the test media. Seeded plates were incubated at 26 ± 0.1°C for 15 days. Then the colony diameter was recorded and percent inhibition was calculated according to the method of Kulkarni and Lingappa (2001).

$$\text{Percent inhibition} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

In the case of plant extracts, after pouring the molten sterilized SMYA into the sterile plates, 0.1 ml of 1% of plant extract was added and the plate was shaken well in order to mix the plant products with the media. 100 ml of respective solvent was also used as control. The culture inoculation and growth inhibition bio-assay was followed as mentioned above. Compatible inhibition effect of the plant extracts and plant based insecticides with fungicide were found out using the following formula:

$$\text{Compatible efficacy (CE)} = 100 - \frac{\text{Fungal colony growth in fungicide treatment}}{\text{Colony growth in other treatments}} \times 100$$

Dual liquid assay

The dual liquid assay was also carried out to determine the compatibility of the plant products with the entomopathogenic fungi. 100 ml of SMYEB in 250 ml of conical flask was inoculated with 0.1 ml of the fungal spore suspension and the recommended dosage of commercially available plant products separately. Control was maintained for each treatment and the inoculated flasks were incubated at 26 ± 0.1°C for 15 days in BOD incubator (Remi, Mumbai). After 15 days, the mycelial mat was taken out from the flask by using sterile spatula and placed in the Petri dishes containing filter paper. The initial weight of the paper was recorded. The Petri dishes were kept in hot air oven at 50 ± 1° C for one hour and the dry weight of the fungal mycelia was recorded. The inhibitory activity was assessed by the difference between the dry weight of fungal mycelia in the control and the respective treatment.

Table 1. Influence of commercial botanicals and crude plant extracts on fungal growth (mg) by dual liquid bioassay method.

Treatments		*B. bassiana*	*I. fumosoroseus*	*L. lecanii*
Commercial products				
T_1	Biospark	201.3 ± 0.1*	176.3 ± 0.2*+	194.7 ± 0.1*+
T_2	Phytophrate	196.5 ± 0.3*	173.4 ± 0.3*+	191.3 ± 0.2*+
T_3	Exodos	198.3 ± 0.1*	169.3 ± 0.1*+	183.7 ± 0.1*+
T_4	Biodos	197.2 ± 0.4*	163.4 ± 0.4*+	186.1 ± 0.2*+
T_5	Neemgold	206.2 ± 0.4*	180.4 ± 0.2*+	193.7 ± 0.1*+
T_6	Control	219.24 ± 0.1*	191.4 ± 0.3*+	207.4 ± 0.1*+
Plant extracts				
T_7	*S. nodiflora* (Benzene)	209.7 ± 0.2*	176.7 ± 0.3*+	187.3 ± 0.3*+
T_8	*S. nodiflora* (Methanol)	207.2 ± 0.3*	171.2 ± 0.4*+	183.2 ± 0.1*+
T_9	*V. negundo* (Methanol)	190.7 ± 0.3*	169.4 ± 0.3*+	179.3 ± 0.3*+
T_{10}	*V. negundo* (Water)	199.2 ± 0.1*	179.7 ± 0.3*+	189.7 ± 0.1*+
T_{11}	*A. squomosa* (ethanol)	170.3 ± 0.4*	150.4 ± 0.2*+	171.4 ± 0.3* ^{N}
T_{12}	*A. squomosa* (Water)	191.4 ± 0.1*	170.1 ± 0.4*+	177.2 ± 0.2*+
T_{13}	*I. carnea* (Methanol)	201.6 ± 0.4*	181.6 ± 0.4*+	191.7 ± 0.1*+
T_{14}	*I. cornea* (Water)	210.7 ± 0.1*	182.7 ± 0.4*+	195.4 ± 0.2*+
T_{15}	*P.tomentosa* (benzene)	206.3 ± 0.1*	179.7 ± 0.2*+	196.4 ± 0.1*+
T_{16}	*P.tomentosa* (ethanol)	204.1 ± 0.3*	176.1 ± 0.4*+	194.2 ± 0.4*+
T_{17}	*P. aquililum* (Hexane)	201.4 ± 1.2*	169.7 ± 0.1*+	176.1 ± 0.4*+
T_{18}	Canbendazim	20.4 ± 0.3	21.01 ± 0.1 ^{N}	20.10 ± 0.2 ^{N}

*Turkey multiple range test analyses between fungicide to other treatments at 5% level; analyzes between *B. bassiana* to *P. fumosorosea* and *V. lecanii* separately denoted by +, N indicates not significant.

Statistical analysis

The experimental design for all trials was completely randomized. The data were analyzed using the analysis of variance (ANOVA) and the mean values were compared by using the Turkey multiple range test ($P \leq 0.05$) using statistical package for the social sciences (SPSS) package 11.5 version.

RESULTS AND DISCUSSION

Commercial botanicals

The growth inhibition of *B. bassiana* by phytophorate (16.73%) and biodos (16.85%) revealed that these two products were compatible with this fungus (Table 1). A similar trend was also observed in dual liquid assay (Table 2). However, the compatible efficacy (CE) analyses show that plant-based products were compatible between 84.5 to 85.7% for *B. bassiana*; 84.0 to 85.9% for *I. fumosorosea* and 84.6 to 86% for *V. lecanii* in colony growth methods. Similar compatibility was also recorded in the dry weight method (86.2 - 86.8, 83.6 -85.5 and 85.6 - 86.7 for *B. bassiana, I. fumosorosea* and *L. lecanii,* respectively). The mycelial dry weight of *B. bassiana* was 201.3, 196.5, 198.3, 197.2 and 206.2 mg on biospark, phytophrate, exodos, biodos and neemgold treatment, respectively, whereas the control recorded 219.2 mg. The growth inhibition of *L. lecanii* was recorded as 13.48, 14.36, 17.46 and 18.27 for biospark, phytophrate, neemgold and biodos treatment, respectively. Exodos recorded 20.25% growth inhibition. Statistical analysis between *B. bassiana* to *I. fumosorosea* and *B. bassiana* to *L. lecanii* indicated that they were statistically significant at 5% by Turkey test. Similarly, the statistical comparison between controls to plant-based insecticides was also significant ($P < 0.05$). *I. fumosoroseus* and *L. lecanii* could also be well tolerated to the plant products.

All the commercial plant based pesticides were well tolerated by *B. bassiana.* Neemgold and biospark were relatively very safe followed by exodos (15.46%). The action mechanism of neem by-products on vegetative growth and reproduction of fungi is still unknown (Locke, 1995). However, phytoalexines, sulfurade compounds and triterpenoids in these products have fungitoxic action (Singh et al. 1984; Bandopadhyay, 2002). Babu et al. (2001) also studied the toxicity of neem seed kernel extract (NSKE) and combination of NSKE and the entomopathogenic fungus *Beauvaria bassiana* on *S. litura* under laboratory trials. They observed that the combination of NSKE with *B. bassiana* significantly increased the mortality than the individual treatments. Vyas et al. (1992) reported that, neemark, a biopesticide of neem was well tolerated by another important fungus *Metarhizium anisopliae*. Devi and Prasad (1996) also found that neem

Table 2. Impact of commercial botanicals and crude plant extract on colony growth of fungal pathogen (in mm ± SE) by dual plate bioassay method.

Treatment		*B. bassiana*	*I. fumosoroseus*	*L. lecanii*
Commercial products				
T_1	Biospark	61.3 ± 0.4 *	63.2 ± 0.1*+	64.2 ± 0.1*+
T_2	Phytophrate	59.2 ± 0.3 *	62.1 ± 0.4 *+	63.5 ± 0.1*+
T_3	Exodos	60.1 ± 0.1*	60.1 ± 0.1 *N	59.1 ± 0.1* N
T_4	Biodos	59.1 ± 0.5*	57.1 ± 0.1 * N	60.6 ± 0.3*+
T_5	Neemgold	63.1 ± 0.4*	64.1 ± 0.1 *N	61.2 ± 0.1*+
Plant extracts				
T_6	*S. nodiflora* (Benzene)	63.8 ± 0.1*	61.8 ± 0.1*+	59.1 ± 0.4*+
T_7	*S. nodiflora* (Methanol)	61.4 ± 0.3*	60.2 ± 0.1* N	60.2 ± 0.1 * N
T_8	*V. negundo* (Methanol)	59.4 ± 0.1*	59.1 ± 0.2* N	58.1 ± 0.1* N
T_9	*V. negundo* (Water)	61.4 ± 0.3*	64.2 ± 0.1*+	66.1 ± 0.4*+
T_{10}	*A. squomosa* (Methanol)	49.1 ± 0.1*	50.2 ± 0.2* N	54.2 ± 0.1*+
T_{11}	*A. squomosa* (Water)	57.1 ± 0.1*	58.1 ± 0.2* N	60.1 ± 0.3*+
T_{12}	*I. carnea* (Methanol)	59.1 ± 0.1*	63.1 ± 0.1*+	61.7 ± 0.1* N
T_{13}	*I. carnea* (Water)	63.2 ± 0.1*	67.4 ± 0.1 *+	68.5 ± 0.4*+
T_{14}	*P. tomentosa* (Benzene)	62.1 ± 0.1*	66.1 ± 0.1*+	69.2 ± 0.4*+
T_{15}	*P. tomentosa* (Methanol)	61.5 ± 0.1*	65.3 ± 0.2*+	70.1 ± 0.1*+
T_{16}	*P. aquilinum* (Hexane)	60.1 ± 0.2*	60.4 ± 0.4 * N	61.2 ± 0.4* N
T_{17}	Canbendazim	09.1 ± 0.2	09.1 ± 0.1 N	09.1 ± 0.1 N
T_{18}	Control	71.1 ± 0.3*	72.3 ± 0.3* N	74.2 ± 0.4*+

*Turkey multiple range test analyses between fungicide to other treatments at 5% level; analyzes between *B. bassiana* to *P. fumosorosea* and *V. lecanii* separately denoted by +, N indicates not significant.

and pongamia were well tolerated by *Nomuraea rileyi*. Furthermore, all commercial formulations were commonly used for the control of both sucking and defoliator insects throughout India.

Plant extracts

All the tested plant extracts were found to be compatible with *B. bassiana* except *A. squomosa* (Table 1) (30%), but the water extract recorded only 19.6% inhibition. The other plant extracts were also found to be compatible in the same assay. Similarly, the dual liquid assay also revealed an inhibitory effect of *A. squosoma*, *B. bassiana*, *I. fumosorosea* and *L. lecanii,* respectively (170.3, 150.4 and 170.4 mg) (Table 2). As observed in *B. bassiana,* methanol extract of *A. squomosa* also inhibited the growth of *P. fumosoroseus*. Methanol extract of *V. regundo* had more impact on *P. fumosoroseus*, *I. carnea* and water extracts were highly compatible with *I. fumosoroseus*. Dual liquid assay showed similar impact on the tested fungi.

Benzene and methanol extracts of *P. tomentosa* and water extract of *I. carnea* exhibited 07.5, 06.6 and 05.4% inhibitory activity, respectively. *A. squomosa* methanol extract caused 26.75% growth inhibition followed by methanol extract of *V. negundo* and *S. nodiflora.* All the tested fungi less tolerated hexane extract of *P. aquilinum* and water extracts of *A. squomosa*. Similar inhibitory effect was also observed in liquid assay. The fungicide carbendazim exhibited the highest fungi static property to all the three tested pathogens (88.75, 87.36 and 87.70% for *B. bassiana*, *I. fumosorosea* and *L. lecanii*, respectively). When the fungicide zone of inhibition was compared with other treatments, it was very clear from the results that other treatments caused only 13.00 to 16.82% inhibition in *L. lecanii*, 14.33 to 15.20 in *B. bassiana* and 10.79 to 15.13 in *I. fumosoroseus.*

In both methods, *A. squomosa* ethanol extract highly and significantly reduced the growth of all the entomopathogenic fungi. *L. lecanii* and *I. fumosorosea* growth was highly suppressed by respectively, in both methods. Irrespective of the bioassay methods, neemgold and biospark had the least impact on both *B. bassiana* and *I. fumosorosea* and *L. lecanii*, respectively. These commercial biopesticides can be utilized for pest management along with these microbial insecticides. Rogerio et al. (2005) recorded compatability of neem extracts with *B. bassiana.*

Laboratory compatibility tests have the advantage of exposing the pathogen to the maximum activity possible of chemical products and or plant-based products, a situation that does not occur under field conditions. Therefore, when a treatment is compatible *in vitro*, there is a strong evidence of its selectivity under field conditions. However, a high toxicity *in vitro* does not mean that the

product will always be toxic for that pathogen in the field (Alves et al., 1998). In this situation, inhibition of vegetative growth might be a less representative indication of fungi toxicity than the viability of spores or the effect on germination (Loria et al., 1983). Consequently, because the commercial formula does not take into account the effect of treatment on spore viability, research results suggest precaution when using neem emulsifiable oil in environments where *B. bassiana* affects tested insect mortality significantly. Under field conditions, compatibility between the plant protection product and spore germination is necessary because insects become infected by means of spore germination, by ingestion or contact (Malo, 1993). Hirose et al. (2001) observed 45% reduction in spore germination of *B. bassiana* when mixed with neem oil at 2%. *B. bassiana* activity was enhanced by agroneem (a commercial neem insecticide) (Al-Mazraaw et al., 2009) as observed in our study. Borgio et al. (2008) suggested that plant products can be used along with *M. anisopliae* (Metsch.) Sorok.

Biological control, particularly by entomopathogenic fungi, is important for reducing the population density of pests in IPM programs. Therefore, preservation of entomopathogens that occur naturally, or are introduced for insect control, should be observed (Oliveira et al., 2003). In addition, we must understand the compatibility of entomopathogenic fungi with other crop production techniques such as the use of insecticides, which may inhibit to a smaller or larger extent the development and reproduction of pathogen (Malo, 1993; Rogério et al., 2005). In this context, this study also provides useful information on the compatibility between the fungal biological control agents and plant-based insecticides and plant extracts which are normally used in pest management. This study also reveals that the mixture of either commercial botanicals or plant extracts and fungicides can be used for field application along with these fungi. Moreover, tested botanicals and fungi have been used in pest management and these components can be integratively used in BIPM.

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