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COMPATIBILITY OF ENTOMOPATHOGENIC FUNGI, *METARHIZIUM ANISOPLIAE* AND *PAECILOMYCES FUMOSOROSEUS* WITH SELECTIVE INSECTICIDES

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Abstract

Investigations were carried out to evaluate influence of some selective insecticides on mycelial growth and conidial germination of *Metarhizium anisopliae* (Metsch.) Sorokin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith. All insecticides significantly inhibited mycelial growth and conidial germination of the fungal pathogens. Lorsban was the most toxic insecticide to mycelial growth and conidial germination followed by Lannate, Larvin and Pirate. Cascade, Match, Steward and Proclaim were comparatively less toxic to mycelial growth (36.78-48.67% inhibition) and conidial germination (40.32-49.97% inhibition) of the fungal pathogens. Conversely, Runner, Capture, Abamectin and Curacron were compatible with significantly lesser inhibition in growth (25.19-36.47%) and conidial germination (27.78-43.66%) of the fungi. Tracer was found safe to conidial germination and growth of the fungi.

Introduction

Entomopathogenic fungi have considerable potential for efficacious suppression of a variety of arthropod pests. However, their field application may give inconsistent control because infections of hosts with insect pathogenic fungi are easily affected by abiotic factors like temperature & humidity and biotic factors like interactions of antagonistic microorganisms (Ferron, 1978; Villani *et al*., 1992). Furthermore, fungi cannot replace need for chemical insecticides in all commercial agro ecosystems. Insecticides are always required to suppress rapidly expanding insect pest populations. Strategies have been employed to increase efficiency and accelerate insect mortality by combining entomopathogenic fungi with sub lethal doses of chemical insecticides and botanicals. There are numerous examples where applications of selective insecticides have enhanced the efficiency of entomopathogenic fungi against insect pests (Quintela & McCoy 1998; Dayakar *et al*., 2000; Serebrov *et al*., 2005; Purwar & Sachan, 2006). However, potential inhibitory effects of pesticides on entomopathogenic fungi cannot be ignored. Variations in toxicity response of entomopathogenic fungi from synergistic, antagonistic or neutral to insecticides have been observed (Mietkiewski & Gorski, 1995; Gupta *et al*., 1999). Fungal biological control agents and selective insecticide may act synergistically increasing the efficiency of the control, allowing the lower doses of insecticides, preservation of natural enemies, minimizing environmental pollution and decreasing the likelihood of development of resistance to either agent (Boman, 1980; Moino & Alves, 1998; Ambethgar, 2009). By contrast, use of incompatible insecticides may inhibit growth and reproduction of the pathogens and adversely affect integrated pest

management (Duarte *et al*., 1992; Malo, 1993). Hence, an understanding of effects of synthetic insecticides on germination and vegetative growth of fungal biocontrol agents is essential. Many experiments have been carried out to investigate effects of insecticides on various developmental stages of entomopathogenic fungi (Li & Holdom, 1994; Er & Gokce, 2004; Alialzadeh *et al*., 2007; Rachappa *et al*., 2007).

Most fungus-insecticide compatibility studies have dealt with the effects of insecticides on mycelial growth and sporulation of fungi. Whereas, effects of insecticides on conidial germination, that have been ignored frequently, is the most important aspect to evaluate insecticide compatibility (Neves *et al*., 2001; Hirose *et al*., 2001) since it is the first step of the infection process (Oliveria *et al*., 2003).

The potential inhibitory effects of pesticides on germination and mycelial growth of entomopathogenic fungi often vary among fungal species and strains (Vanninen & Hokkanen, 1988; Anderson *et al*., 1989). Therefore, fungal genotypes compatible to particular pesticides can be identified and manipulated. The present investigations were taken up to evaluate the effects of various insecticides (Table 1) on mycelial growth and conidial germination of *Metarhizium anisopliae* (Metsch.) Sorokin and *Paecilomyces fumosoroseus* (Wize) Brown & Smith in *In vitro*.

Materials and Methods

Fungus culture: The fungal pathogen, *Paecilomyces fumosoroseus* (n32) used in present study was obtained from IPM laboratory Plant protection institute, South China University, China. *Metarhizium anisopliae* (L6) was of local origin. These fungi were cultured on potato dextrose agar medium (PDA) autoclaved at 121°C (15 Psi) for 15-20 minutes and poured into sterilized Petri plates. The Petri plates containing PDA medium were incubated at 27 ± 1°C, 80 ± 5% relative humidity and photophase of 12 hours. The conidia were harvested gently by scraping the surface of 15-days old culture with inoculation needle. The conidia were suspended in distilled water containing 0.1% Tween-80. The mixture was stirred on a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using haemocytometer. Suspension of desired concentration (1×10^6 conidia ml^{-1}) was prepared in distilled water containing 0.1% Tween-80 and was preserved at 5°C until used in bioassay.

Growth inhibition test: Each insecticide based on field application rate (Table 1) was added to the PDA medium (100 ml) in flask before solidification (medium temperature 48°C) to get desired concentration and was mixed thoroughly. The medium was then poured equally into the five Petri plates. Small disc (5 mm dia.) of young fungal mycelium was cut with sterile cork borer and placed aseptically in the centre of each plate containing the poisoned medium. Petri plates were incubated at 27 ± 1°C, 80 ± 5% relative humidity and photophase of 12 hours. For each treatment four replications were maintained. Check without poison was kept for comparison under the same conditions. Fungal colony diameter was measured with a caliper rule 10 days after inoculation and compared with standard check to measure the degree of toxicity of different insecticides used in the study. Percent growth inhibition of each fungal isolate over untreated check was worked out for the respective insecticides.

Table 1. Insecticides used in bioassays.

S. No.	Insecticides tested		Recommended dose g or ml/ acre
	Trade name	Common name	
1.	Lorsban® 40EC	Chlorpyriphos	1000
2.	Lannate® 40 SP	Methomyl	250
3.	Larvin® 80 DF	Thiodicarb	300
4.	Pirate® 360 SC	Chlorfenapyr	320
5.	Steward® 15 EC	Indexacarb	175
6.	Proclaim® 1.9 EC	Emamectin Benzoate	200
7.	Match® 50EC	Lufenuron	200
8.	Curacron® 50 EC	Prophenophos	1000
9.	Abamectin 1.8 EC	Abamectin	400
10.	Capture® 20 SC,	Triflumuron	200
11.	Cascade® 10 DC	Flufenoxuron	200
12.	Runner® 240 SC	Methoxyfenozide	200
13.	Tracer® 240 SC	Spinosad	80

Germination inhibition test: Each insecticide based on field application rate (Table 1) was added to water (100 ml) in flask to get desired insecticide suspension. The effect of insecticides on the germination of the conidia was determined by placing 10 micro liter drop of each insecticide suspension containing fungal spores at concentration of 1 x 10^6 conidia ml^{-1} on a thin film of PDA medium in a Petri plate. The conidia in distilled water suspension served as control for comparison. The experiment was conducted under CRD with four replications of each treatment. Petri plates were incubated at 27 ± 1°C, 80 ± 5% relative humidity in the dark for 24 hours. After staining with lacto-phenol cotton blue, germination was checked under microscope. Only conidia with a germ tube as long as the conidium widths were considered to have germinated. Inhibition of conidial germination over untreated check was worked out for the each insecticide.

Statistical analysis: Data were subjected to analysis of variance (Statistix 9) and means were separated by the Tukey HSD test at p=0.05

Results

***Metarhizium anisopliae*:** All insecticides tested caused highly significant inhibition in the growth (6.84-72.45%) of *M. anisopliae* (F = 46.51, df = 12, p<0.0001). Lorsban was found the most effective (P = 0.05) in retarding the fungus growth significantly over others, followed by Lannate, Larvin and Pirate (Fig. 1). Proclaim, Steward and Cascade were found moderately toxic to the fungus by retarding fungal growth from 43.20 to 48.67%. Growth inhibitions caused by Proclaim and Steward were statistically at par. Conversely, Capture, abamectin, Curacron and Match were found compatible with comparatively less detrimental effects (33.49 to 40.11% inhibition) on the fungal growth. Significantly less growth inhibition was recorded in Runner (25.19%). Tracer was found safe to the fungus growth by inhibiting only 6.84% growth.

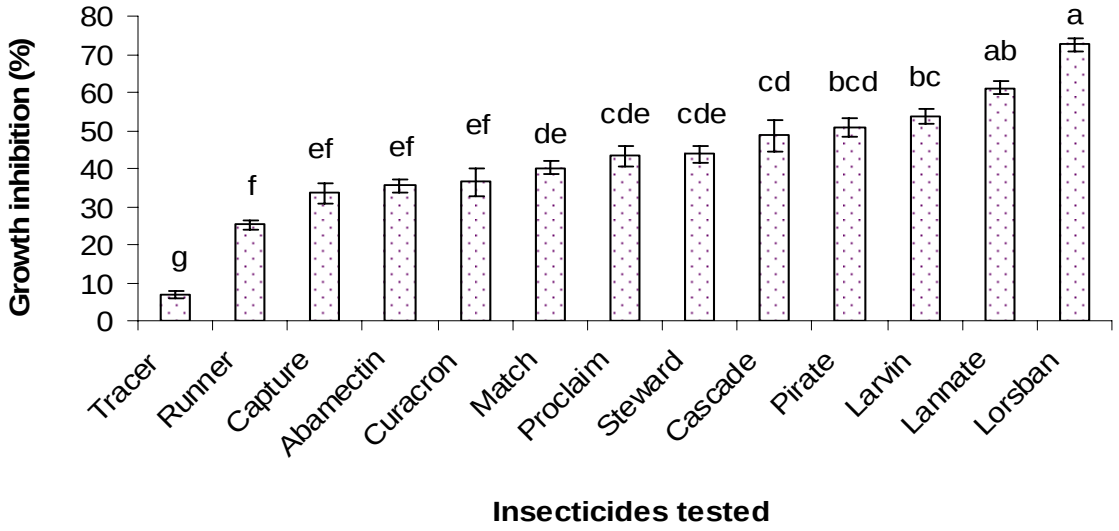

Fig. 1. Effect of various insecticides on mycelial growth of *Metarhizium anisopliae*.
Means followed by different letters are significantly different from each other according to Tukey HSD Test at p=0.05. Bars represent Standard error of means based on four replications.

Germination inhibition of the fungal conidia also varied (19.35-83.99%) significantly among insecticides (F = 58.52, df = 12, p<0.0001). Tracer was the least toxic to the fungal conidial germination significantly different from all treatments (Fig. 2). On the other hand, Lorsban was the most toxic to conidial germination of the fungus followed by Lannate, Larvin and Pirate with germination inhibition of 68.28, 60.67 and 55.98%, respectively. Abamectin, Curacron, Match, Cascade, Steward and Proclaim were comparatively less toxic (41.60-49.97% inhibition) to the conidial germination. Germination inhibitions caused by Curacron, Match and Cascade were statistically at par. Runner and Capture were least toxic (30.94-35.04% inhibition) to the fungal conidial germination among insect growth regulators (IGRs) tested.

***Paecilomyces fumosoroseus*:** The toxicity of various insecticides to mycelial growth of *P. fumosoroseus* is presented in Fig. 3. The growth reduction (8.30-74.80%) of the fungus differed significantly among the insecticides (F = 34.68, df = 12, p<0.0001). Lorsban caused the highest growth inhibition significantly over others, followed by Lannate, Larvin, Proclaim and Pirate. Fungal growth inhibition by Steward (41.96%) and Match were statistically same. Curacron, Capture, Runner, Cascade and abamectin were compatible with comparatively less inhibition in growth (29.40-37.73%) of the fungi. Capture and Runner were statistically at par. Similarly, growth inhibitions caused by Cascade and abamectin were also without statistical difference. Tracer was found nearly safe to the fungus growth with only 8.30% growth inhibition.

Insecticides also caused highly significant inhibitions in the conidial germination of the fungus (F = 67.99, df = 12, p<0.0001). Lorsban with germination inhibition of 85.43% (Fig. 4) had the most adverse effect on the fungal conidial germination, followed by Lannate (70.04%), Larvin and Pirate. Cascade, Abamectin, Match, Proclaim and Steward were found moderately toxic (40.32-49.74% inhibition) to conidial germination of the fungus. Conversely, Runner, Curacron and Capture were found less detrimental (27.78 to 34.34% inhibition) to conidial germination of the fungal isolate whereas, Tracer was the least toxic (20.82% inhibition) to conidial germination of the fungus among insecticides tested.

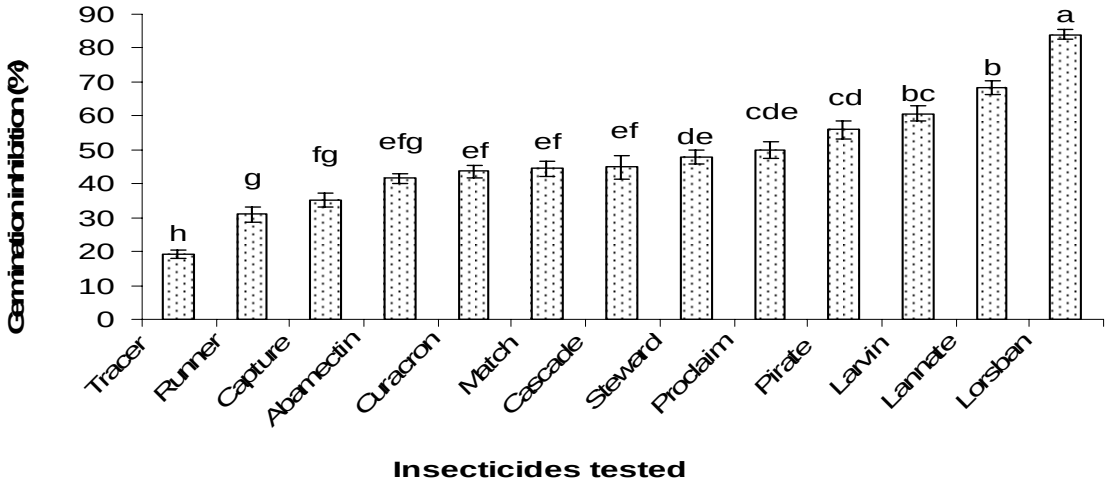

Fig. 2. Effect of various insecticides on germination of *Metarhizium anisopliae*. Means followed by different letters are significantly different from each other according to Tukey HSD Test at p=0.05. Bars represent Standard error of means based on four replications.

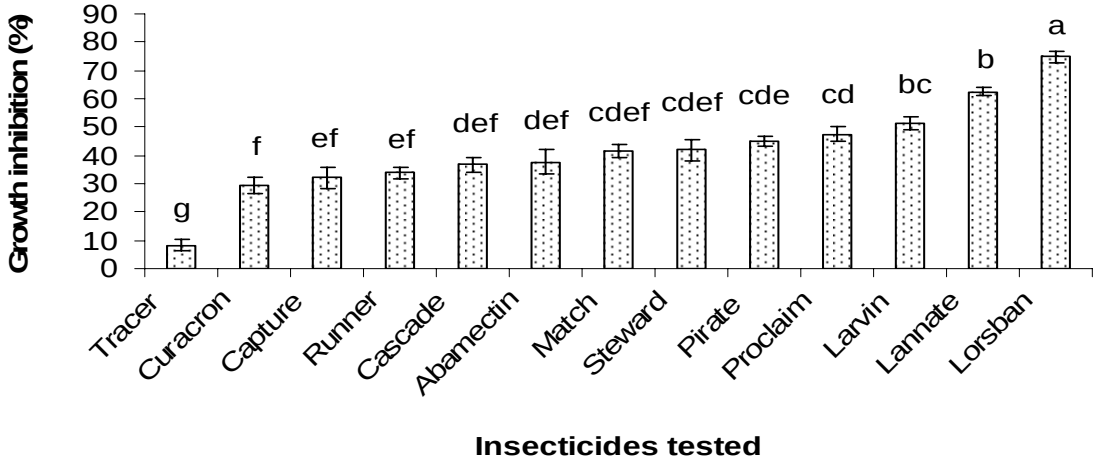

Fig. 3. Effect of various insecticides on mycelial growth of *Paecilomyces fumosoroseus*.
Means followed by different letters are significantly different from each other according to Tukey HSD Test at p=0.05. Bars represent Standard error of means based on four replications.

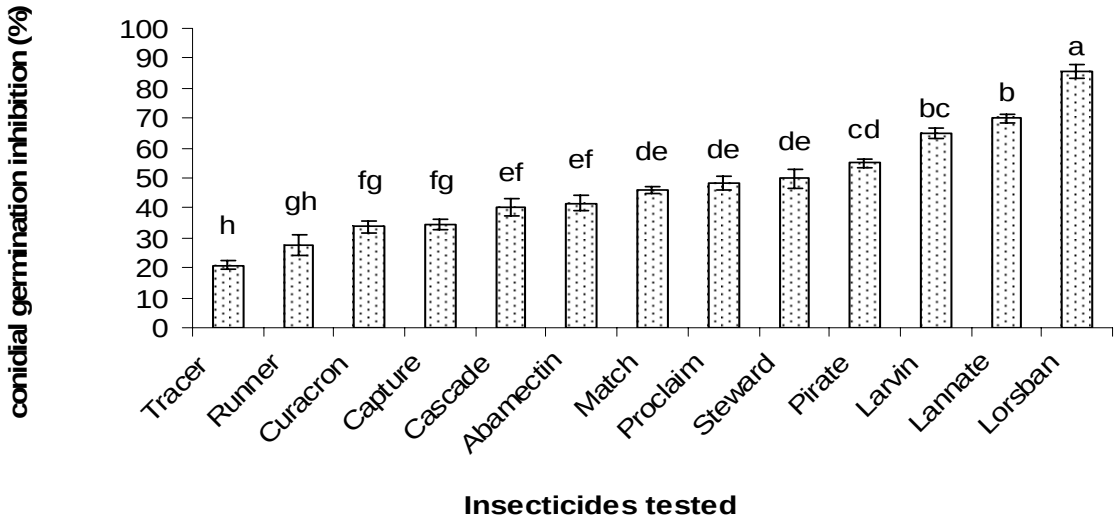

Fig. 4. Effect of various insecticides on germination of *Paecilomyces fumosoroseus*.
Means followed by different letters are significantly different from each other according to Tukey HSD Test at p=0.05. Bars represent Standard error of means based on four replications.

Discussion

Insecticides have potential to affect the various developmental stages of entomopathogenic fungi. All tested insecticides displayed varying degree of potential to inhibit growth and conidial germination of both entomopathogenic fungi corroborating previous findings of Mietkiewski & Gorski (1995) and Gupta *et al*., (1999). They observed variations in toxicity response of entomopathogenic fungi from synergistic, antagonistic or neutral to insecticides. Hassan & Charnley (1989) also reported inconsistent interaction between fungus and insecticides.

These investigations revealed that Lorsban (chlorpyriphos) was the most detrimental to the both entomopathogenic fungi. Lannate, Larvin and Pirate were moderately toxic. IGRs were less detrimental to the growth and germination of the fungi. Tracer was found safe to both fungi. Mohammad *et al*., (1987) and Rachappa *et al*., (2007) reported similar findings. They observed extremely detrimental effects of chlorpyriphos to various developmental stages of *M. anisopliae* while methomyl was moderately toxic. Li & Holdom (1994) also demonstrated extremely toxic effects of Lorsban on growth and sporulation of *M. anisopliae*. Indexacarb and Profenophos have been investigated to be less detrimental to the fungi while insect growth regulators and Spinosad have been found comparatively safe to the fungi (Rachappa *et al*., 2007). The observed variations in the inhibitory potential could be due to inherent variability of chemical insecticides to entomopathogenic fungi. Their inhibitory potential varies both between and within chemical classes (Inglis *et al*., 2001). Fungitoxic effects of insecticides vary as a function of the chemical nature of the products and interacting microbial species (Antonio *et al*., 2001; Kumar *at al*., 2008). A given insecticide may have different fungitoxic effects on various developmental stages of the fungus (Li & Holdom, 1994). The potential inhibitory effects of pesticides on germination and mycelial growth of entomopathogenic fungi vary among taxa and strains (Vanninen & Hokkanen, 1988; Anderson *et al*., 1989).

Effect of insecticides on conidial germination is the most important aspect to evaluate fungus-insecticide compatibility (Neves *et al*., 2001; Hirose *et al*., 2001). This happens because the fungi infect insects through the conidial germination which is the first step of the infection process (Oliveria *et al*., 2003). Our results suggested that conidial germination was more sensitive to insecticides than myceliel growth of the fungi. The gemination of conidia is more severely affected than growth of entomopathogenic fungi in presence of pesticides (Hall, 1981; Er & Gokce, 2004). The differences observed between germination and growth inhibition are probably due to some reduction over time of the insecticide's effect in PDA medium, since germination was assessed 1 day post treatment while radial growth was measured 10 days post treatment. Griffen (1994) observed that effects of many insecticides on fungus growth decline gradually over time.

We suggest that except a few (Lorsban and Lannate) all other insecticides tested can be used along with the entomopathogenic fungi. Tracer, Runner, Curacron, Capture and abamectin are comparatively more compatible with the fungi. However, results may differ in field because fungi are exposed maximum to insecticides *In vitro* which doesn't occur under field conditions. Additionally, fungi may recover after some chemical pesticides are decomposed on plant leaves. Therefore, once an insecticide is proved to be compatible in the laboratory, it must be selective under field conditions. On the other hand, high *In vitro* toxicity of a product will not always be same in the field (Butt & Brownbridge, 1997) but is likely to occur (Alves *et al*., 1998).

Present investigations showed complex and varying effects of insecticides on the fungi, their actual effects on the fungi at cellular as well as 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 insecticides can be employed in combination with the fungi for insect control under field conditions.

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