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Research Article

INTERACTIVE EFFECT OF ENTOMOPATHOGENIC FUNGI *PAECILOMYCES FUMOSOROSEUS* WITH FEW ORGANOPHOSPHATE AND PYRETHROID PESTICIDES: AN IN VITRO STUDY

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ABSTRACT
To be effective as biopesticide, the entomopathogenic fungal conidiation and its germination should occur effectively in the field condition where they use as plant protective agent. This is affected by a number of environmental factors. Therefore, we have studied in vitro compatibility of *Paecilomyces fumosoroseus* (MTCC-4097) in combination with five insecticides @ 250, 500 and 1000ppm using three mycological media such as Saboraud dextrose agar (SDA), Potato dextrose agar (PDA), and Czapek Dox agar (CDA). The data indicate a fair growth of *P. fumosoroseus* in Saboraud dextrose broth than Potato dextrose broth and Czapek Dox broth. Similarly, the percentage of spore counts was greater in SDA than other two media. Among the five pesticides tested phorate seems to be more toxic to the test organism than other four pesticides (malathion, chloropyrifos, deltamethrin and permethrin). Furthermore, this study also emphasized none of the pesticides studied have shown compatibility with myco-insecticide, *P.fumosoroseus.*

Key Words: *Pesticides, Entomopathogenic Fungi, Compatibility and Mycological Media*

INTRODUCTION
Synthetic chemical insecticides provide many benefits on agricultural crop production and human health, but they also pose certain hazards. An alternative method of insect management offer adequate levels of pest control with fewer hazards. One such alternative is the use of microbial insecticides that contain micro organisms or their by-products. These myco-insecticides (fungal based organisms) are gaining much importance as natural control agents of many insect species, including stored product insects (Carruthers and Hural, 1990). High valuability of microbial insecticides lies because of their extremely low toxicity to non-target animals and human beings. Therefore, bio-pesticides are safe for both the pesticide user and consumers of treated crops, compared to commonly use synthetic chemical pesticides.
Pacilomyces fumosoroseus an entomopathogenic fungus which has a considerable potential for suppression of variety of insect pests. Its field application however, sometimes give inconsistent control. This is because the infections of host with entomopathogenic fungi are easily affected by number of factors such as temperature, humidity and also interaction of antagonistic organisms (Ferron, 1978; Villani *et al.,* 1992). Nevertheless, insecticides are always in demand to suppress fast growing insect populations in the field. Therefore, fungi cannot replace need for chemical insecticides in all commercial agricultural crops. However, strategies have been developed to increase its efficacy and accelerate insect mortality by combining entomopathogenic fungi with sublethal doses of chemical insecticides. Number of studies have made where use of selective pesticidal chemicals have increased the efficacy of entomopathogenic fungi against insect pests (Quintela and Mc Coy, 1998; Dayakar *et al.,* 2000; Serebrov *et al.,* 2005; Purwar and Sachan, 2006). Despite the synergistic activity of an entomopathogenic fungus and certain agrochemical (pesticides) combinations, the potential inhibitory effects of these two cannot be ignored. Furthermore, there is varied response of toxicity of entomopathogenic fungi viz., synergistic, neutral or antagonistic to insecticides have been reported (Mietkiewski and Gorski, 1995; Gupta *et al* .,1999). Boman, (1980); Moino and Alves, (1998); Ambethgar, (2009) have reported synergistic activity

of entomopathogenic fungi along with few insecticides which has increased its effective control, allowing the low doses of insecticides, minimizing environmental contamination and decrease the likelihood of development of resistance to either agent. On the other hand, incompatible insecticides used in crop protection might inhibit the growth and reproduction of the myco-pathogen which would inturn affects the integrated pest management (Duarte *et al.,* 1992; Malo, 1993). Hence, it is very essential to know the effect of chemical pesticides on germination potential and vegetative growth of fungal biocontrol agent. Most of the studies done on entomopathogenic fungi and insecticide compatibility revealed, influence of pesticides on mycelial growth and sporulation of the fungus; whereas effect of spore germination with chemical pesticides had been disregarded, although it is an important aspect in evaluating the pesticide compatibility, since it is a prerequisite in the process of host infection (Oliveria *et al.,* 2003). As such, in evaluating the effects of pesticides on a microbial control agent, these traits need to be considered (Keller, 1994). Keeping this in view, the present study was conducted using five pesticides (phorate, malathion, chloropyrifos, deltamethrin and permethrin) to evaluate their effect on spore germination and growth of *Paecilomyces fumosoroseus* in-vitro.

MATERIALS AND METHODS

Media preparation, culture inoculation and conidial estimation

Standard PDA Medium (Hi-media Ltd., Mumbai) was autoclaved at 120^0C for 15 minutes, cooled to 40 ± 5^0C, and then amended with 0.3g/L of streptomycin sulphate. The required concentrations (250, 500, 1000 ppm) of pesticides 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. After solidification, 10days old culture of *P. fumosoroseus* was point inoculated at the centre of the plates. All triplicate sets were then incubated at 25 ± 1^0C for nine days. At the end of incubation, the colony area was measured and a central disc (1cm) was drawn from each treatment to quantify the conidial concentration. For this estimation, a standard sample colony (without pesticide) area against pesticide treated colony area was chosen from control plate. Each disc was placed in a sterile test tube and conidia were suspended in 10ml of water containing 0.02% Tween-80, vortexed for 2 minutes. The concentration of conidia was estimated under the compound microscope (Olympus, Japan) using a neubauer haemocytometer.

Biomass Production

Triplicate sets of 100 ml sterile potato dextrose broth (PDB) supplimented with 0.3g/L of streptomycin sulphate was dispensed into 250 ml conical flasks. To this, desired concentration of pesticides and 1ml of fungal spore suspension containing $1x10^6$ spore/ml was added aseptically. Control flasks (without pesticides) were also run along with treated flasks. All inoculated flasks were incubated at 25 ± 0.1^0C for 10 days in BOD incubator (Ind Lab., Chennai). At the end of incubation, the mycelial mat was separated by Whatman ^{TM} filter paper No.1 and dried at 80 ± 1^0C for over-night to achieve constant dry weight.

Disc Inoculation

One ml of required concentration (250, 500, 100ppm) of different pesticides was added to the sterile petri dishes. Following which, cooled mycological media (PDA, SDA and CDA about $40^0\pm5^0$C) was added to the plates and the plates were agitated aseptically to get a uniform distribution of the pesticides. The plates were allowed to solidify in the laminar air flow for 30 minutes. A young fungal colony (5 days old) of *P. fumosoroseus* was cut with sterile cork borer (8mm dia.) and placed aseptically in the centre of each petri plate containing poisoned medium. Control plates without pesticides were also maintained. All in triplicate sets were incubated at 25 ± 1^0 C for one week and the colony diameter was measured and recorded.

Statistical Analysis

The data obtained from various experiments were statistically analyzed and subjected to Least Significant Difference (LSD).

RESULTS

Compatibility of *P. fumosoroseus* with five chemical pesticides (three OP compounds and two synthetic pyrethroids) on vegetative growth and colony development in three mycological media by disc inoculation on PDA, SDA and CDA are given in Table-2.

Table 1: Pesticides, their IUPAC name and the concentrations used

Pesticides	IUPAC Name	Concentration (ppm)
Phorate	O,O-Diethyl-s-(ethyl thio methyl) phosphorodithioate	250 500 1000
Malathion	Diethyl 2- (dimethoxyphosphorothioyl sulphanyl) butanedioate	250 500 1000
Chloropyrifos	O,O- diethyl o-3,5,6-trichloropyridin-2-ylphosphorothioate	250 500 1000
Deltamethrin	(S)-cyano-(3-phenoxyphynyl)-methyl (1R,3R)-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate	250 500 1000
Permethrin	3-Phenoixybenzyl(1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate	250 500 1000

The data indicate maximum growth (5.1cm) in control PDA plates than SDA and CDA. Further, phorate was least compatible to *P.fumosoroseus* than other four pesticides as it has shown very poor colony growth at 500 and 1000 ppm levels (2.8 and 2.9cm dia, respectively). However, moderate growth (3.1-4.0mm dia) of the organism was observed in PDA plates treated with other four pesticides such as melathion, permethrin, deltamethrin and chloropyriphos. Unlike in PDA, the growth of *P. fumosoroseus* in SDA was not significantly different (P<0.05%) than the control. In this medium the fungus could establish relatively fair growth in almost all pesticide treated plates, including phorate (3.7-3.9cm dia). The colony development in CDA was almost similar to that of PDA in which phorate at higher concentration (500 and 1000 ppm) was more detrimental to the organism (Table-2) than any other pesticides.

Mean score in a column with different letters are significantly different at P< 0.05 by Least significant difference (LSD).

Table-3 shows the results on biomass production of *P. fumosoroseus* in pesticide treated liquid medium (PDB, SDB and CDB). The results revealed a fair growth of the organism in SDB than PDB, although it has not supported the growth in toto compared to control. However, the growth pattern of *P. fumosoroseus* in CDB is similar to that of PDB, in which significant reduction (P<0.05%) in biomass production was observed in pesticide treated flasks. Among the three mycological liquid media the fungus had shown relatively good growth in SDB than other two media (PDB and CDB).

Mean score in a column with different letters are significantly different at P< 0.05 by Least Significant Difference (LSD).

Table 2: Compatibility of *Paecilomyces fumosoroseus* with different pesticides: Vegetative growth and colony development in three mycological media (Disc Inoculation)

Treatments (in ppm)	Potato Dextrose Agar (PDA)	Sabouraud's Dextrose Agar (SDA)	Czapek Dox Agar (CDA)
PHORATE			
250	3.9^{de}	3.9^{ab}	3.4^{b}
500	2.8^{a}	3.8^{ab}	1.9^{a}
1000	2.9^{ab}	3.7^{ab}	1.6^{a}
MALATHION			
250	3.9^{de}	3.3^{a}	5.0ef
500	3.7^{cde}	3.6^{ab}	4.9^{ef}
1000	3.6^{cde}	3.6^{ab}	5.0^{ef}
CHLOROPYRIFOS			
250	4.0^{e}	3.9^{ab}	5.0^{ef}
500	3.4^{abcde}	3.8^{ab}	5.0^{ef}
1000	3.2^{abc}	4.0^{b}	4.8^{def}
DELTAMETHRIN			
250	3.7^{cde}	4.1^{b}	5.0ef
500	3.5^{bcde}	3.9^{ab}	4.7^{def}
1000	3.1^{abc}	3.9^{ab}	3.9^{bc}
PERMETHRIN			
250	3.5^{bcde}	3.7^{ab}	4.7^{def}
500	3.3^{bcd}	3.8^{ab}	4.5^{cde}
1000	3.2^{abc}	3.6^{ab}	4.2^{cd}
CONTROL	5.1^{f}	4.06^{b}	4.0^{bc}

Effect of different pesticides on percentage of spore count of *P. fumosoroseus* is shown in Table-4. In general, the percentage of spore counts was higher in PDA and SDA than CDA. It appears that phorate is more toxic than other four pesticide treatments as it has shown less percentage of spore counts in all three mycological media under study. Contrary to this, the other four pesticides viz., malathion, permethrin, deltamethrin and chloropyriphos at 250ppm levels have shown >90% spore counts both in PDA and SDA. Out of the three mycological media studied for percentage of spore counts of *P. fumosoroseus*, only SDA has recorded higher percentage in all three concentrations, although >90% spore counts were recorded at 250 ppm of pesticides (except phorate)treated in PDA.

DISCUSSION

Information on compatibility of entomapathogenic fungi with chemical pesticides is needed because it provides valuable data useful for the development of strategies for handling plagues in organic agriculture. In this direction, the work carried out in the present investigation on vegetative growth

indicated a significant reduction in colony growth of *P. fumosoroseus* in all pesticide treated plates of PDA. Inversely, no significant reduction was observed with SDA and to some extent in CDA. Of the five

Table 3: Synergistic activity of different pesticides on biomass production of *Paecilomyces fumosoroseus* in three liquid media

Treatments (in ppm)	Potato Dextrose Broth (PDB)	Sabouraud's Dextrose Broth (SDB)	Czapek Dox Broth (CDB)
PHORATE			
250	0.43^{cde}	1.43^{abcd}	0.73^{d}
500	0.40^{bcd}	1.46^{cd}	0.37^{c}
1000	0.29^{abcd}	1.40^{abcd}	0.30^{bc}
MALATHION			
250	0.66^{f}	1.5^{d}	0.31^{bc}
500	0.66^{f}	1.44^{abcd}	0.29^{abc}
1000	0.40^{cd}	1.24^{ab}	0.14^{a}
CHLOROPYRIFOS			
250	0.64^{ef}	1.43^{abcd}	1.38^{e}
500	0.19^{ab}	1.43^{abcd}	1.37^{e}
1000	0.18^{a}	1.37^{abcd}	0.22^{ab}
DELTAMETHRIN			
250	0.44^{de}	1.38^{abcd}	0.26^{abc}
500	0.29^{abcd}	1.34^{abcd}	0.17^{ab}
1000	0.25^{abcd}	1.35^{abcd}	0.14^{a}
PERMETHRIN			
250	0.32^{abcd}	1.45^{bcd}	0.19^{ab}
500	0.24^{abcd}	1.27^{abc}	0.17^{ab}
1000	0.22^{bc}	1.23^{a}	0.16^{ab}
CONTROL	0.77g	1.56^{e}	0.72^{cd}

pesticides tested, phorate was more toxic than other four pesticides. Marzieh Rashid *et al.,* (2010) reported drastic reduction in conidial germination of *M.anisopliae* when fipronil, pyriproxyfen and hexaflumuron were amended to SDA at the highest concentration (0-15%). They also found higher negative effects with hexaflumuron than other two pesticides (fipronil and pyriproxyfen) indicating its noncompativeness together with Metarhizium anisopliae. In the present investigations among the five insecticides tested, phorate was more detrimental to the test fungus both on solid and liquid medium. Similarly, the study made on percentage of spore counts on *P. fumosoroseus* indicated least spore count in phorate treated petri plates than other four pesticides. These results demonstrate, most of the pesticides

studied here have shown non compativeness with the test fungus, although there was a marginal increase in vegetative growth of the organism in malathion and chloropyriphos amended plates of CDA (Table-2).

Table 4: Influence of pesticides on percentage of spore count with three mycological Media

Treatments (in ppm)	Potato Dextrose Agar (PDA)	Sabouraud's Dextrose Agar (SDA)	Czapek Dox Agar (CDA)
PHORATE			
250	62.57^{d}	62.20^{a}	49.85^{cde}
500	83.49^{f}	78.33^{b}	47.30^{cd}
1000	99.42^{j}	52.27^{a}	32.35^{b}
MALATHION			
250	99.51^{j}	99.95^{e}	68.97^{h}
500	98.37^{ij}	94.06^{cde}	68.26^{gh}
1000	58.86cd	84.11^{bcd}	60.90^{fg}
CHLOROPYRIFOS			
250	94.25^{hi}	96.72^{e}	82.15^{i}
500	86.79^{fg}	88.50^{bcde}	46.36^{cd}
1000	78.11^{e}	82.60^{bc}	42.77^{c}
DELTAMETHRIN			
250	90.79^{gh}	96.46^{de}	52.097^{de}
500	58.45^{cd}	94.20^{cde}	51.03^{de}
1000	34.29^{a}	89.34^{bcde}	20.67^{a}
PERMETHRIN			
250	98.80^{ij}	99.87^{e}	57.24^{ef}
500	54.40^{bc}	98.99e	55.85^{ef}
1000	53.60^{b}	77.30^{b}	42.20^{c}

Hassan and Charnely, (1989) also observed inconsistent interactions between chemical insecticides and entomopathogenic fungi. In another study made by Li and Holdam, (1994) have shown chlorinated hydrocarbon insecticides are more toxic to entomopathogens than other insecticides. They reported that carbamate insecticides such as carbofuran, methoxyl and oxymyl were moderately toxic while chloropyriphos, malathion and tempephos were extremely toxic. Integrated pest management (IPM) has its own limitations as incompatible pesticides might lead to inhibition of development and multiplication of entomopathogens (Anderson and Robert, 1983; Duarte *et al.*, 1992; Malo, 1993). Alves *et al*., (1998) reported when the insecticide is compatible in vitro, there are strong evidence about its selectivity under field conditions. Similarly, higher toxicity in vitro does not always means that the same phenomena will occur in the field, but it indicates such possibility. Chemical insecticides cause varied levels of inhibition of vegetative growth and spore germination of entomopathogen, depending on concentration of active

ingredient and chemical nature. In our study, phorate has shown maximum negative effect on vegetative growth and sporulation of *P. fumosoroseus.* Data obtained by Li and Holdam, (1995) while working with acetic acid, citric acid and malic acid indicate, reduced vegetative growth and sporulation of *M.anisopliae*. Ghini and Kimati, (2000) emphasized that organophosphate (OP) compounds interfere directly on membrane permeability of the cells and synthesis of enzymes, consequently affecting the metabolic process of the organism. As a result, it inhibits the enzyme that converts phosphatydilethanalamine into chitin. Probably, the same mechanism of inhibition was responsible for drastic reduction of *P. fumosoroseus* sporulation and vegetative growth in this study. In conclusion, the present study highlights neither pyrethroids nor OP compounds under study are compatible to use in organic agriculture, like insecticide together with *P. fumosoroseus* in IPM management.

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