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COMPATIBILITY OF AGROCHEMICAL WITH ENTOMOPATHOGENIC FUNGI (*PAECILOMYCES LILACINUS*) – A BIOLOGICAL NEMATICIDE

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Abstract

The present study attempts have made to study the effect of some fungicide such as blue copper (copper oxy chloride 0.3%), difenoconazole(0.07%), propiconozale(0.05%) and herbicides like gramaxone(0.5%) and fernoxone(0.2%) with *Paecilomyces lilacinus*-an entomopathogenic fungus which is a biological nematicide.The optical density values, serial dilution method, and radial growth technique were carried out to find the compatibility of some agrochemicals with the entomopathogenic fungi-*Paecilomyces lilacinus*.The concentration of the agrochemical used under field recommendation was not compatible. The study indicated that this concentration highly inhibit the growth of the fungus. If we reduce the concentration of the agrochemical the fungi may grow. Final result says that the fungicide and herbicide are non-compatible with this fungus. Further study to be needed to find the compatibility level at low concentration.

Key words: Fungicide, Herbicides, Entomopathogenic fungus *Paecilomyces lilacinus*.

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INTRODUCTION

Herbicides are widely used in agriculture. We use chemical herbicides in agriculture. First used herbicide was 2.4-dichlorophenoxyacetic acid. Some are known to be mutagenic and carcinogenic [1]. The use of fungicides in agriculture, to protect plants from soil-borne pathogens, is a common practice [2]. However, there is a dearth of information on the side-effects of fungicides on key soil ecological processes. The fungicide-treated soils in general had less microbial biomass N concentrations than the untreated soils. Effects of the fungicides on soil nutrient cycling processes were not large and were specific to each fungicide [3].

There are about 20,000 species of nematodes. Some are beneficial nematodes. Plant parasitic nematodes damage the plants by feeding on the roots either externally or internally. Environmental concerns over conventional nematicides have led to increasing interest in the use of biological control agents to control plant-parasitic nematodes [4]. The development of nematophagous fungi as biological control agents has revealed a need for further understanding of their infection processes. The egg-parasitic fungi, *Pochonia chlamydosporia* and *Paecilomyces lilacinus*, and the nematode trapping fungus, *Arthrobotrys oligospora*, have received the most attention [5]. Through the application of biochemistry and molecular biology, aspects of their infection processes have been elucidated.

Paecilomyces lilacinus is typically isolated from soil, particularly in samples originating from warmer regions. *It* is a cosmopolitan filamentous fungus which inhibits the soil, decaying plants and food products.*Paecilomyces lilacinus* is a common saprobic, filamentous fungus [6]. *Paecilomyces lilacinus* is known to directly parasite the eggs and other stages development of common plant infecting nematodes [7]. Fungal spores germinate and the mycelium invades the nematode.A basic serine protease enzyme was produced by the fungus which can act against *Meloidogyne hapla* eggs (root –knot) of nematodes. They weaken the egg shell and kill the nematodes [8]. Effective against plant parasitic nematodes, including root-knot (*Meloidogyne species*) burrowing (*Radophakus*) similes *(cob*) and cyst (*Globadera species and Heterodera species*) nematodes [9].

MATERIALS AND METHODS

The pure culture of *Paecilomyces lilacinus* was subcultured in the laboratory in selective medium Potato carrot agar (PCA) by maintaining it for 3-5days at room temperature (24-28°C) to get sufficient quantity of inoculums.

Prepare six 100 ml lots of PCA media. It was sterilized in an autoclave at 15psi for 20minutes.After sterilization the 100ml lots of PCA medium was inoculated with 1ml pure culture of *Paecilomyces lilacinus* fungi along with the agrochemicals at respective concentration and incubated for 5days at 24°C.

TREATMENTS

TREATMENTS		DOSAGE
T1	-	**Blue Copper (0.3%) +*Paecilomyces lilacinus***
T2	-	**Difenoconazole (0.07%) +*Paecilomyces lilacinus***
T3	-	**Propiconazole (0.05%) +*Paecilomyces lilacinus***
T4	-	**Gramoxone (0.5%) +*Paecilomyces lilacinus***
T5	-	**Fernoxone (0.2%) +*Paecilomyces lilacinus***
T6	-	***Control (Paecilomyces lilacinus)***

REPLICATION: Two replication were maintained.

Optical Density: The sample T1, T2, T3, T4, T5 and T6 was transferred to the cuvettes after incubation of 24hours, 48hours.72hours, 96hours, 120hours and 144hours the optical density values were recorded. The results were tabulated and data was prepared according to the final observation. The absorbance of all the samples was taken at 570nm using UV-spectrophotometer.

***Spore Count (Serial Dilution Method)*:** From the above respective treatment 1ml of broth was taken for serial dilution. The treated sample after 144 hours completion of Optical Density it was used for Serial Dilution Method. The initial dilution was prepared by adding 1ml of the sample into a 9ml dilution blank labeled 10^{-1}.From the first dilution 1ml of the suspension from 10^{-1} was transferred to the dilution blank 10^{-2} thus the serial dilution 10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} were made by pipetting 1ml into dilution blanks containing 9ml of sterile distilled water serially as adapted customarily.1ml of various dilutions was added to sterile Petri plates, 15ml of sterilized cold molten (45°C) PCA media was added, and 10^{-8} and 10^{-9} were selected for enumeration. Replication (R1 and R2) are maintained for each of the treatments and control also maintained. Plates were incubated invertedly for 3-5days at room temperature, fungal growth to the said culture medium.

***Radial Growth (Poison Food Technique)*:** The different concentrations of agrochemicals were used for treatment. Prepare 100ml lots of PCA medium and sterilize in an autoclave at 15psi for 20minutes. Incorporate the respective agrochemicals like Blue copper (0.3%), difenoconazole (0.07%), propiconazole (0.05%), gramaxone (0.5%) and fernoxone (0.2%) in to the medium separately at warm condition and mix well.Pour the medium in sterile petriplates and allow for solidification. Control plates also maintained without agrochemicals. Replication (R1 and R2) were maintained for each of the treatments.With the use of sterile cork borer cut a small fungal culture from the preliminary maintained culture plate. Place the culture on the centre of the solidified petriplates and incubated at room temperature (24°C).Incubation after 24hours, 48hours, 72hours, 96hours, 120hours, and 144hours the radial growth was measured in centimeters and the results were predicted.

RESULTS AND DISCUSSION

Optical Density: *The* optical density value at 24hours, 48hours, 72hours, 96hours, 120hours, and 144hours was compared with the control. The fungicide Blue copper (0.3%) showed 2.1 optical density at 144hours. Whereas control showed 1.4 optical density. Difenoconazole (0.07%) and propiconazole (0.05%) showed 0.1 and 0.33 optical density at 144hours when compared to the control the optical density reduced. The herbicide gramaxone (0.5%) and fernoxone (0.2%) also showed 0.72 and 0.59 optical density in *Table 1*. There was a gradual decrease of all the agrochemicals except blue copper.

***Spore Count (Serial Dilution Method)*:** The percentage of inhibition was calculated by counting the colonies on the different treatment plates with control plates.Spore count was taken from 10^{-8} dilution

and 10^{-9} dilution.The control plates showed more colony forming units per ml of culture. The dilution of 10^{-8} plate showed 93 colonies and dilution of 10^{-9} showed 28 colonies. This was compared with the treatment plates. The fungicide blue copper (0.5%), difenoconazole (0.07%), propiconazole (0.05%) plates did not show any colony morphology.The herbicide gramaxone (0.5%) also did not show any colony morphology on the plates. but the herbicide fernoxone (0.2%) showed 7 colonies at 10^{-8} dilution plates and 4 colonies at 10^{-9} CFU/ml in *Table 2 & Figure 1.*

CALCULATION

The percentage of inhibition was calculated by using the formula,

$$\text{Percentage of inhibition} = \frac{\text{No. of CFU in control plate - No. of CFU in treated plate}}{\text{Number of colonies in control plate}} \times 100$$

$$\text{Per centage of inhibition of the treated Agrochemical fernoxone at } 10^{-8} \text{ dilution} = \frac{93-7}{93} \times 100$$

$$= 0.924 \times 100$$

$$= 92.47\%$$

$$\text{Percentage of inhibition of the treated Agrochemical fernoxone at } 10^{-9} \text{ dilution} = \frac{28-4}{28} \times 100$$

$$= 0.857 \times 100$$

$$= 85.71\%$$

The percentage of inhibition of fernoxone at 10^{-8} dilution was 92.47% and 10^{-9} dilution was 85.71%.

***Radial Growth (Poison Food Technique)*:** The radial growth of the entomopathogenic fungus *Paecilomyces lilacinus* was taken for each treatment. The control plate showed radial growth at 24 hours (0.6cm) and it gradually increased at 48 hours (1.4cm), 72 hours (1.8cm), 96 hours (2.1cm), 120 hours (2.3cm) and 144hours (2.4cm). This was compared with the different treatment plates. This technique was done to confirm the present study.The fungicide blue copper at the concentration of 0.3%, difenoconazole at 0.07% and propiconazole at 0.05% showed no radial growth when compared with control plate at 24-144hours.The herbicide gramaxone at the concentration of 0.5% did not show any radial growth, but the herbicide fernoxone at the concentration of 0.2% showed radial growth at 24hours (0.5cm) ,48hours (1.2cm), 72hours (1.7cm), 96hours (1.9cm), 120hours (2cm) and 144hours (2.2cm). When it compared with control plate, the radial growth gradually decreased in fernoxone.The radial growth was measured at 24 hours till 144 hours and the values showed that the herbicide fernoxone was compatible to the fungus (*Paecilomyces lilacinus*). Radial growths indicate that respective concentration is not compatible to the fungi *is showed in Table 3 & Figure 2.*

CONCLUSION

The studies have showed that the compatibility of *Paecilomyces lilacinus* a well known fungal antagonist with commercially available chemical fungicides and herbicides against root-knot disease especially as soil applications.

The present study indicates the fungicide Blue copper (0.3%), Difenoconazole (0.07%), Propiconazole (0.05%) and herbicide gramaxone (0.5%) showed no growth in colony forming unit and also in radial growth method.Eventhough the optical density value was showed high in Blue copper the growth was not found in confirmative studies. So this density may be due to the chemical deposited. These agrochemicals are not compatible with field level recommendation. The present study was compared with the primilanary studies. Concluded that herbicides are less toxic than fungicide when they studied the compatibility with the fungi *Paecilomyces lilacinus*.such that, the present study must be further continued by using low concentration of agrochemicals.

TABLE: 1, Compatibility of Agrochemicals with *Paecilomyces lilacinus* by Optical Density Method

Treatments	OPTICAL DENSITY					
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
T1 - Blue copper (0.3%)	1.37	1.61	1.45	3.1	1.3	2.1
T2 – Difenoconazole (0.07%)	0.92	0.27	0.77	0.02	0	0.1
T3 – Propiconazole (0.05%)	1.31	0.47	-0.09	-0.49	-0.44	-0.33
T4 – Gramaxone (0.5%)	0.53	0.44	0.4	0.43	0.5	0.72
T5 – Fernoxone (0.2%)	0.36	0.99	1.24	1.19	0.77	0.59
T 6 – Control	0.06	1.13	1.38	1.47	1.6	1.47

TABLE: 2, Compatibility of Agrochemicals with *Paecilomyces lilacinus* at 10^{-8} and 10^{-9} dilution

TREATMENTS	SPORE COUNT	
	10^{-8} dilution	10^{-9} dilution
T1 - Blue copper (0.3%)	NIL	NIL
T2 – Difenoconazole (0.07%)	NIL	NIL
T3 – Propiconazole (0.05%)	NIL	NIL
T4 – Gramaxone (0.5%)	NIL	NIL
T5 – Fernoxone (0.2%)	7	4
T 6 – Control	93	28

Percentage of inhibition on the growth of *Paecilomyces lilacinus* with agrochemicals

TREATMENTS	SPORE COUNT in CFU/ml	
	10^{-8} dilution	10^{-9} dilution
T1 - Blue copper (0.3%)	100%	100%
T2 - Difenoconazole (0.07%)	100%	100%
T3 - Propiconazole (0.05%)	100%	100%
T4 - Gramaxone (0.5%)	100%	100%
T5 - Fernoxone (0.2%)	92.47%	85.71%
T 6 – Control	-	-

TABLE: 3, Compatibility of Agrochemicals with *Paecilomyces lilacinus* by Radial Growth Method

Treatments	RADIAL GROWTH in cm					
	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
T1 - Blue copper (0.3%)	NIL	NIL	NIL	NIL	NIL	NIL
T2 – Difenoconazole (0.07%)	NIL	NIL	NIL	NIL	NIL	NIL
T3 – Propiconazole (0.05%)	NIL	NIL	NIL	NIL	NIL	NIL
T4 – Gramaxone (0.5%)	NIL	NIL	NIL	NIL	NIL	NIL
T5 – Fernoxone (0.2%)	0.5	1.2	1.7	1.9	2	2.2
T6 – Control	0.6	1.4	1.8	2.1	2.3	2.4

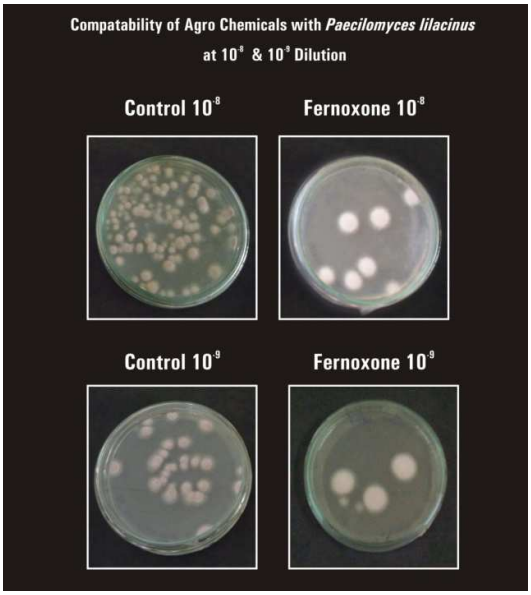

Figure 1

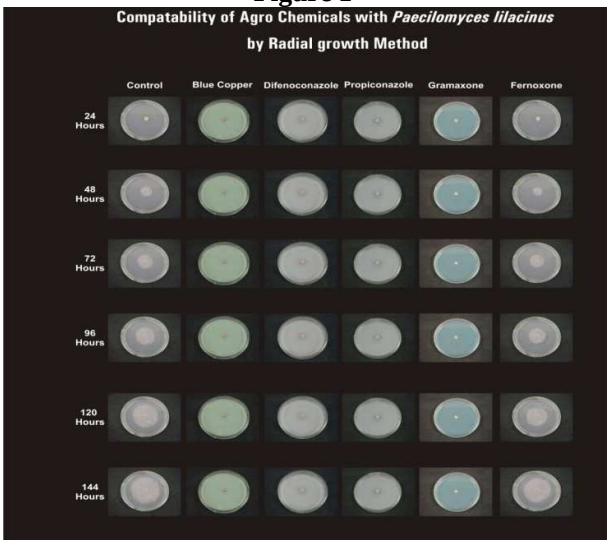

Figure 2

REFERENCES

[1] Shu-Kang Chen, Clive A, Edwards, & Subler S, 2001, Soil Ecology Laboratory.
[2] Larone, D.H, 1995, Medically important fungi-A guide for identification of fungal diseases, Washington, D.C.
[3] Ragupathy, A., Palanisamy, S., Chandramohan, N., Gunathilagaraj, K, 1997, **A** Guide on Crop Pest, 4, 51 - 195.
[4] Villanueva, L.M., Davide, R.G, 1986, Annual Review of Phytopathology, 24, 453-489.
[5] Walia, R.K., Vats, R, 2000, Critical Reviews in Plant Sciences, 1(19), 1 - 30.
[6] Park,J. O.S. Hargreaves, J.R. , McConville, E.J. , Stirling, G.R., Ghisalberti, E.L. & Sivasithamparam, K, 2004, The Society for Applied Microbiology, 271 – 276.
[7] Rita J. Holland, Keith L. Williams, and K. M. Helena Nevalainen, 2004, *Paecilomyces lilacinus* strain Bioact251 is not a plant endophyte**.**
[8] Dong, L.Q., Yang, J.K and Zhang, K.Q, 2007, *Journal of Applied Microbiology*, 6 (103), 2476 – 2488.
[9] Kiewnick, S, 2007, *J Nematol*, 1(39), 67–104.