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IN VITRO EVALUATION OF SOME ANTAGONISTS AND PLANT EXTRACTS AGAINST *PYTHIUM APHANIDERMATUM* CAUSES DAMPING OFF OF CHILLI

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ABSTRACT
Selected plant extracts and bio-control agents were tested against *Pythium aphanidermatum* causes damping off of chilli. The plant leaf extract of *Lantana camara*at (20%) concentration showed maximum inhibition of mycelial growth of *Pythium aphanidermatum* (98.87 %)followed by *Eucalyptus globolus* (20%) with(93.61%)inhibition. Among the bio-control agents *Trichoderma harzianum* showed maximum inhibition (98.20%)of the test pathogen followed by the*Trichoderma viride* (88.89%). Fungicide ridomil also tested for comparison with biocontrol and botanicals and it shows 99.64% inhibition over the control at 200 ppm concentration.The *in vitro* studies revealed that botanicals *Lantana camara* (20%) and *Eucalyptus globolus* (20%) shows highest mycelial growth inhibition over the control.

INTRODUCTION

Chilli (*Capsicum annum* L.) is an important tropical and subtropical crop of India as well as of world. It is rich source of vitamin C, A and B. In India it is an important commercial crop as India is an International market largest producer and exporters of chilli, chilli powder and dry chilli. Although India has largest growing area, chilli productivity is relatively low. The prominent reason for this is the high incidence of fungal and viral diseases.

Chilli crop is attacked by several diseases those leading to great loss of cultivars. Among those diseases, damping off of chilli incited by *Pythium aphanidermatum*(Edson) Fitz caused more than 60% mortality of seedling in both nurseries and field grown crops either as pre or post-emergence damping off in nurseries and fields (Jadhav and Ambadkar, 2007). The most common mean to manage this disease to use fungicides. However, use of fungicide has adverse effect on soil health and public health by residual toxicity of soil, and environmental pollution. It also decimates non target and beneficial microorganisms. Development of fungicidal resistant has also been reported in some variety of crops (Bharathi *et al*., 2004).

Recently the researcher focused on developing environmentally safe, effective biocontrol agent and plant products. *Pseudomonas fluorescens* and *Trichoderma viride* have been tested against several crop diseases (Lokesha and Benagi, 2007; Sarma *et al.,* 2009). Utilization of plant extract which are natural source of antimicrobial substances, considered as safe and degraded by natural microorganism; and not have any adverse effect on health or environment at any concentrations which they were used (Kim et al., 2004; Yang *et al*., 2010).Now days the management of damping off diseases are mainly based on the fungicides and the adverse effect of these fungicides on environment and human health have observed. Now in this trial the efforts on developing environmental safe, long lasting and effective bio-control agent has been focused. In the view of above the objective of this work was to evaluate the fungi toxic effect of some plant extract, bio-control agents against *Pythium aphanidermatum* causes damping off disease of chilli.

MATERIALS AND METHODS

Isolation of pathogen and maintenance

The pathogen was isolated from diseased seedling of chilli, collected from nursery beds during kharif season,from the experimental field of department of Plant Pathology, SHIATS, Allahabad. Chilli seedlings showing damping off symptoms were collected and collar region washed in running tap water and cut into small pieces. After surface sterilization with (0.1% NaOCl), rinsed in sterile distilled water for 3 times, blotted dry with filter paper and then placed on 2% water agar (Huang and Lin, 1998) and incubated at 25 ± 2^{o}C for 48 hours. Hyphal

tips of fungi growing out from the collar region were transferred on to potato dextrose agar (PDA) medium and maintained in PDA slants (Ainsworth, 1961) and stored at 25 ± 2ºC ina Biological Oxygen Demand (BOD) incubator. The pathogencity of isolated culture was confirmed by Koch's postulates.

Collection of plant material and extraction

Plant leaves of seven plant species used in this study were collected from research field of SHIATS, Allahabad. Extraction of these leaves at three different concentrations 10, 15 and 20 percent were done by the method of Singh and Majumdar (2001). The required plant parts were thoroughly washed with tap water and ground in a mortar and pestle by adding 0.1 M sodium phosphate buffer at the rate of 10 mL/g of leaf tissue . This solution (8 mL) was centrifuged at 9000 rpm for 15 min at 4ºC. The supernatant (5 mL) was collected and sterilized by passing through 0.22 mm lter (Millipore). Each ltrate was preserved as stock 10% solution aseptically in bottles at 5ºC for further use. The plant extracts was diluted further to a 15% and 20% concentration (v/v).

Biocontrol organisms

Soil samples were collected from different places from rhizosphere of chilli plant for the isolation of *Trichoderma* spp. Samples were brought to laboratory and stored at 4ºC until used. Serial dilutions of each soil samples were prepared in sterilized distilled water up to 10^6 and 0.5 ml diluted sample was poured on the surface of Trichoderma Specific Medium (Elad *et al.*,1982). Plates were incubated at 25 ± 2ºC for 96 hours. For the isolation of *Pseudomonas fluorescens* 1g of rhizosphere soil sample was suspended in 10 ml of sterile distilled water. Samples were serially diluted and 100µl of sample was spreaded on King's B (King *et al.*, 1954) plates. After incubation at 28ºC for 48 h the plates were exposed to UV light and the colonies exhibiting the fluorescence were picked up and streaked on to the slants for maintenance. *Bacillus subtilis* was isolated on nutrient agar (NA) medium and maintained on nutrient agar slant at 4ºC.

Efficacy of plant extract

All the botanicals were evaluated in *in vitro* at 10, 15 and 20% concentrations for their fungistatic properties against *Pythium aphanidermatum* by poisoned food techniques (Nene and Thapliyal, 2000). Freshly prepared PDA media was distributed to several conical flasks. Plant extract with 100% concentration was mixed with different volume of PDA media by calculation to make 10, 15 and 20% concentration. The PDA medium mixed with plant extract was poured into sterilized petri plates at 20 ml per plate and allows solidifying. The plates were then incubated 6 mm disc of 4 day old culture of *Pythium aphanidermatum* grown on PDA. These all plates were incubated at 25 ± 2ºC till the growth of the test fungus in control plate was fully covered. The percent inhibition of radial growth of test fungus was calculated by using the formula suggested by Vincent (1947).

$$\text{Percent inhibition (PI)} \quad \frac{C \quad T \quad 100}{C}$$

Where, C and T are the growth in diameter (mm) in control and treatment respectively.

Efficacy of biocontrol agents

Antagonistic effect of seven bioagents was evaluated *in vitro* by using dual culture techniques (Morton and Strouble 1955). Autoclaved and cooled media was poured at 20 mL/plate in (90mm) Petri plates and allow solidify. These plates were incubated with 5 mm disc of 7 days old culture of biocontrol agent as well as 5 mm disc of 4 days old culture of test fungus at equil distance and exactly opposite to the each other on PDA in Petri plates. In the bacterial dual culture, antagonists were streak with the help of sterilized bacterial loop at the end of the media plate. After 24 h of incubation, just opposite of the antagonist bacterial streak, a 5 mm disc of the test pathogen was placed. One disc of test pathogen was placed in the media plate (PDA) in the center served as control. These all plates were incubated at 25 ± 2ºC in the incubator. Observations were recorded as radial mycelium growth of the fungal pathogen and percent inhibition of test pathogen over the control. It was calculated by using formula given by Arora and Upaddhyay (1978) as follow.

$$\text{Percent inhibition (PI)} \quad \frac{C \quad I}{C} \quad 100$$

Where, C and I are the growth in diameter (mm) in control and intersecting plate respectively.

Efficacy of fungicides

The effect of fungicides at different concentrations on the mycelial growth of test fungus was evaluated by applying poisoned food technique (Nene and Thapiyal, 2000). Potato dextrose agar (PDA) was poured in 250 ml capacity sterial conical flask and sterilized at 15 lbs for 15 minutes. The quantity of fungicides for 50, 100, 150 and 200 PPM was calculated and added separately and mixed thoroughly. These fungicides mixed PDA medium with different concentrations of fungicides was poured into the sterilized Petri plates (90 mm) and wait till solidification at room temperature. Then 5 mm disc of one week old culture was cut with the sterilized cork borer, and the disc was aseptically transferred in the centre of Petri plates containing poisoned media with fungicides. One Petri dish with normal PDA was inoculated with test fungus as control. Observations of colonies in diameter were recorded after a week, when control plate filled with test fungus by using the formula of Vincent (1927).

Statistical analysis

In this study Completely Randomized Design (CRD) was adopted. The analysis of variance (ANOVA) technique was applied for drawing conclusion from data. The calculated values were compared the tabulated values at 5% level of probability (Fisher and Yates, 1959) for the appropriate degree of freedom.

RESULTS AND DISCUSSION

Plant extract assay

Aqueous extract of seven plants of seven different plant families were studied and evaluated in *in vitro* conditions for their antifungal activity against *Pythium aphanidermatum* causes damping off of chilli. The result (Table 1) revealed thatall the plant extracts were significantly suppress the growth of test

Table 1: Effect of plant leaf extract on the mycelial growth of *P. aphanidermatum*.

Treatment	Scientific name	Colony diameter (mm)* 10%	15%	20%	Average mean col. dia. (mm)	Inhibition % 10%	15%	20%	Average inhibition (%)
T_0	Control	45.00±00	45.00±00	45.00±00	45.00±00	-	-	-	-
T_1	*Azadirachta indica*	23.50±0.50	20.00±0.50	18.16±0.28	20.55±2.71	46.98	54.88	59.03	54.33
T_2	*Eucalyptus globolus*	14.50±0.50	2.83±0.28	2.66±0.50	6.66±6.78	67.29	93.61	93.99	84.96
T_3	*Catharanthus roseus* L.	23.00±0.28	19.00±0.50	11.83±0.50	17.94±5.65	48.11	57.13	73.31	60.13
T_4	*Lowsonia intermis*	20.50±5.34	18.00±0.50	15.00±0.50	17.83±2.75	53.75	59.39	66.16	60.37
T_5	*Ocimum sanctum*	21.00±0.50	19.16±1.00	17.50±0.29	19.22±1.75	52.62	56.77	60.25	57.28
T_6	*Murraya koenigii*	23.83±0.50	21.83±0.29	15.50±0.29	20.38±4.34	46.24	50.75	65.03	54.71
T_7	*Lantana camara*	13.16±0.29	1.66±0.29	0.50±0.50	5.10±6.99	70.31	96.25	98.87	85.33
	F test	S	S	S					
	S.Ed±	0.49	0.37	1.58					
	CD	1.07	0.80	3.40					

Table 2: Effect of biocontrol agents on the mycelial growth of test fungus pathogen.

Treatments	Mycelial growth (mm)	Per cent reduction over the control
Control	83.25±2.06a	-
Trichoderma viride	09.25±2.53d	88.89
Trichoderma hamatum	11.12±3.19d	86.64
Trichoderma harzianum	01.50±1.77e	98.20
Trichoderma sp.	12.25±1.50d	85.25
Bacillus subtilis	16.62±2.25c	80.03
Pseudomonas fluorescens	19.75±2.02c	76.27
Pseudomonas aeregunesa	26.37±2.28b	68.32
F test	S	
S.Ed±	1.92	
CD	3.46	

Table 3: Effect of chemical fungicides on the mycelial growth of pathogenic test fungus.

Treatments	Mycelial colony growth in diameter (mm) 50 ppm	100 ppm	150 ppm	200 ppm	Average mean col. growth dia.	Inhibition percent 50 ppm	100 ppm	150 ppm	200 ppm	Average inhibition (%)
Control	45	45	45	45	45	-	-	-	-	-
Ridomil	0.33	0.00	0.00	0.00	0.11	99.26	100	100	100	99.81
Carbendazim	16.83	15.50	10.50	5.66	12.12	62.60	65.55	76.66	87.42	73.05
Thiram	20.66	19.00	18.00	15.66	18.33	54.08	57.77	60.00	65.20	59.26
F test	S	S	S	S						
S.Ed±	1.92	1.50	2.47	1.02						
C.D	4.43	3.46	5.71	2.36						

fungus at different concentrations (Fig. 1, 2).

At 20 % concentration of plant extract, *Lantana camara* was most effective treatment and show minimum growth (0.50 mm) of test fungus and inhibition 98.87 % radial growth of test over the control, followed by *Eucalyptus globolus* shows growth of test fungus (2.66 mm) and inhibition 93.99%, *Catharanthus roseus* L. growth of test fungus (11.83 mm) and inhibition 73.31 %, Mehandi shows growth of test fungus (15 mm) and inhibition 66.16 %, Curry leaf shows growth of test fungus (15.50 mm) and inhibition 65.03 %, Tulsi growth of test fungus (17.50 mm) and inhibition 60.25 % and the least affected treatment was *Azadirachta indica* shows growth of test fungus (18.16 mm) and inhibition 59.03 % among all the treatments.

At 15 % concentration of plant extract, *Eucalyptus globolus* was most effective treatment, shows mycelial growth of test fungus (2.83 mm) and inhibit 93.61 % radial growth of test fungus over the control. It was followed by *Lantana camara* shows mycelial growth of test fungus (6.16 mm) and inhibition (86.20 %). The least affected treatment among all treatments at this concentration *Curry leaf* shows mycelial growth of test fungus (21.83 mm) and inhibition 50.75% over the control.

At 10 % concentration of plant extract, *Lantana camara* was most effective treatment and show minimum growth (13.16 mm) of test fungus and inhibit 70.31 % radial growth of test fungus over the control, followed by *Eucalyptus globolus* shows growth of test fungus (14.50 mm) and inhibit 67.29 % of test fungus over the control. *Curry leaf* extract was least affected, shows mycelial growth (23.83 mm) and inhibition 45.24 % over the control.

Average radial fungal pathogen growth and inhibition percent (Table 1)with plant extract tested at different concentrations. All the treatments were significantly reduce the growth of test pathogen over the control but most affected treatment with

Figure 1: Plates showing the effect ofplant leaf extract on the mycelial growth of *P. aphanidermatum* at different concentrations

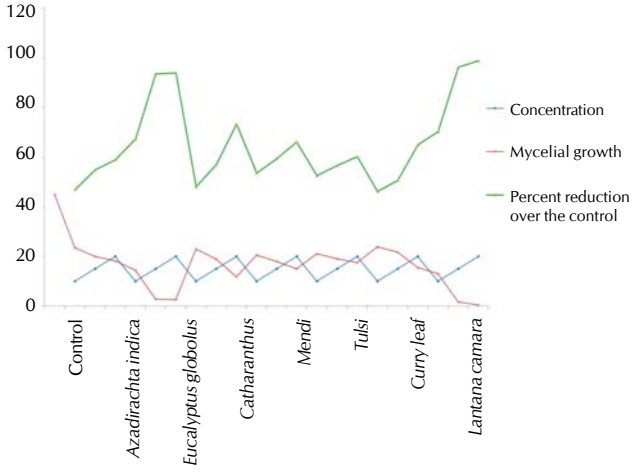

Figure 2: Graphical representation of the effectof plant leaf extract on the mycelial growth of test pathogen at different concentration

lowest growth of test fungus (6.60 mm) and highest inhibition 85.33% was recorded with *Lantana camara* followed by *Eucalyptus globolus,* growth of test fungus (6.66 mm) and inhibition 85.20%.

Least affected treatment among all treatment was *Azadirachta indica* (T_1) shows growth of test fungus (20.55 mm) and highest inhibition 54.33%.

Effectiveness *of Lantana camara* as bio-fungicides has already been reported by Al-Rahmah *et al*., 2013 at 10 mg ml^{-1} concentration. Some other researchers have suggested that antimicrobial components of the plant extracts cross the cell membrane interacting with the enzymes and proteins of the membrane and ultimately their death (Pane *et al*., 2011 and Omidbeygi *et al*., 2007).

Biocontrol agent assay

Seven antagonists *viz. Trichoderma viride, T. hamatum, T.harzianum, Trichoderma sp., Bacillus subtilis, Pseudomonas flueroscens, P. aeregunesa* were evaluated *in vitro* against *Pythium aphanidermatum* applying dual culture technique (Morton and Strouble 1955) using PDA basal medium and result obtained are presented in Table 2.

Result reveals that all the biocontrol agents evaluated exhibited (Fig. 3, 4.) antifungal activity against *Pythium aphanidermatum* and significantly inhibited its mycelial growth as compare to control. Among them*T. harzianum* was found most effective with least mycelium growth (01.50 mm) and highest test fungus growth reduction over the control (98.20%) followed by *T. viride* mycelium growth (09.25 mm) reduction over the control (88.89 %), *T. hamatum* mycelium growth (11.12 mm) reduction over the control (86.64%), *Bacillus subtilis* mycelium growth (16.62 mm) reduction over the control (80.03%), *P. fluorescens* mycelium growth (19.75 mm) reduction over the control (76.27%) and *P. aeregunesa* mycelium growth (26.37%) reduction over the control (68.32%) .

The antagonist effect of *Trichoderma* spp., *Bacillus subtilis* and *Pseudomonas* spp. on *P. aphanidermatum* may be because of mycoparasitism, antibiosis, competition and production of volatile substances by biocontrol agents. The finding of this experiment was also supported by earlier finding Muthukumar *et al*. (2011).

Fungicides assay

It is revealed that (Table 3), all the fungicides at 50, 100, 150 and 200 ppm significantly inhibit mycelium growth of test fungus (Fig. 5,6) over the control. It was found that percent mycelial inhibition was increased with the increase in the fungicides concentrations.

At 50 ppm concentration, the most effective chemical fungicide was ridomil with mycelium growth (0.33 mm) percent reduction over the control (99.26 %), followed by carbendazim mycelium growth (16.83 mm) percent reduction over the control (62.60 %) and thiram with mycelium growth

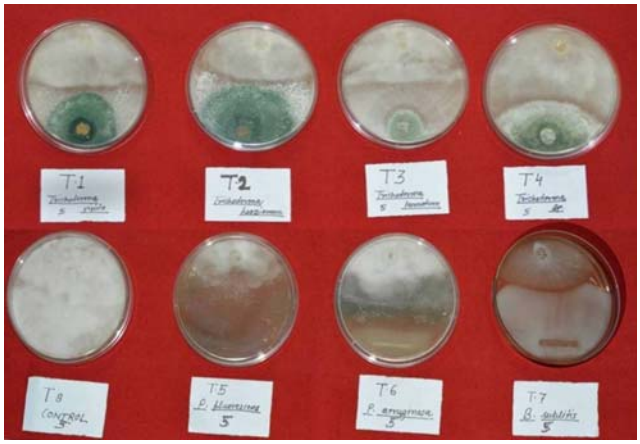

Figure 3: Plates showing the effect of biocontrol agents on the mycelial growth of test fungus pathogen

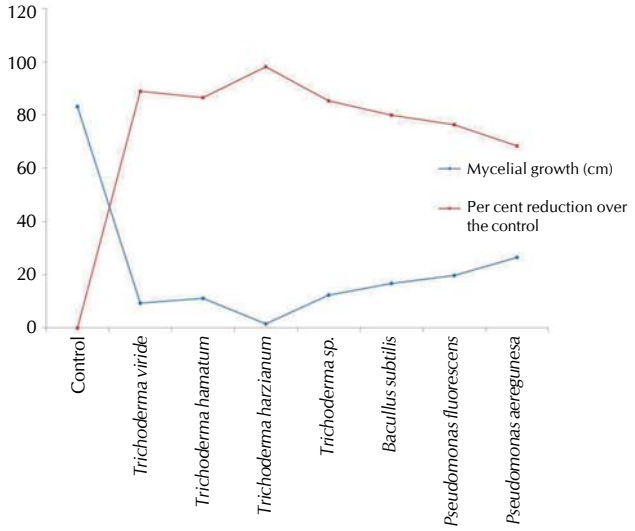

Figure 4: Graphical representation of the effect of biocontrol agents on the mycelial growth of test fungus pathogen

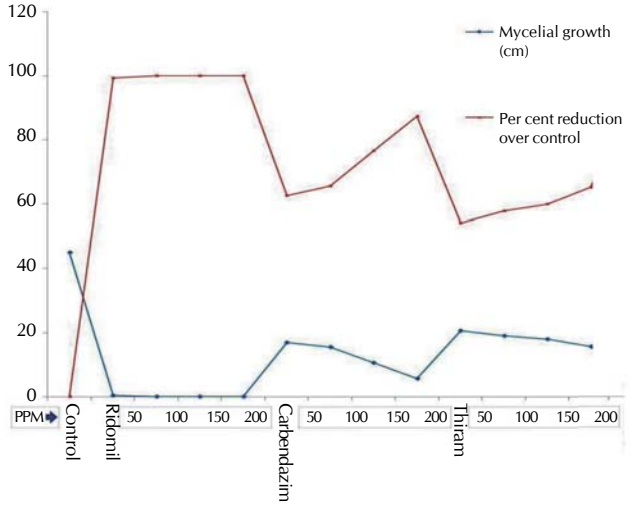

Figure 6: Graphical representation of the effect of chemical fungicides on the mycelial growth of pathogenic test fungus

(20.66 mm) percent reduction (54.08 %) was least effective.

At 100 ppm concentration, the most effective chemical fungicide was ridomil with mycelium growth (0.00 mm) percent reduction over the control (100 %), followed by carbendazim mycelium growth (15.50 mm) percent reduction over the control (65.55 %) and thiram with mycelium growth (19.00 mm) percent reduction (57.77 %) was least effective.

At 150 ppm concentration, the most effective chemical fungicide was ridomil with mycelium growth (0.00 mm) percent reduction over the control (100 %) followed by carbendazim mycelium growth (10.50 mm) percent reduction over the control (76.66 %) and thiram with mycelium growth (18.00 mm) percent reduction (60.00 %) was least effective.

At 200 ppm concentration, the most effective chemical fungicide was ridomil with mycelium growth (0.00 mm) percent reduction over the control (100 %) followed by carbendazim mycelium growth (5.66 mm) percent reduction

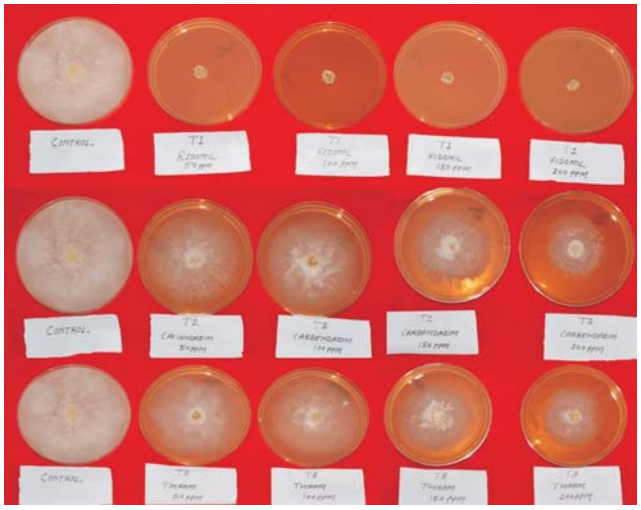

Figure 5: Plates showing the effect of chemical fungicides on the mycelial growth of pathogenic test pathogen at different concentrations

over the control (87.42 %) and thiram with mycelium growth (15.66 mm) percent reduction (65.20 %) was least effective.

Mean percentage effect of all fungicides was recorded revealed that ridomil found most effective with highest mean mycelium inhibition (99.81 %), followed by rest of chemical fungicides. Least effective fungicide was thiram against test pathogen with mycelium inhibition (59.26 %). Similar finding also reported by Suleiman, M. N. (2011) that Ridomil fungicide was the most effective in inhibiting the mycelial growth of *P. aphanidermatum* at all concentrations**.**

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