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Bait Techniques for Isolation of *Pythium aphanidermatum* Causing Damping-off of Chilli from Soil and Efficacy of Bio-agents *In Vitro*

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ABSTRACT

A French bean/ bottle gourd was used as a bait. A bait fruit was transversely cut into 2-4 piece and buried into the infested soil at a depth of 5-6 cm below the soil surface. The soil was irrigated and kept moist. After 24-48 hour observed mycelial growth aseptically transferred to potato dextrose agar media. Eight known bio agents evaluated by two different method *viz*., Pathogen at centre and dual culture techniques. Among them the *Trichoderma harzianum* and *Bacillus subtilis* were most effective antagonist in both methods. Maximum inhibition of mycelial growth of the pathogen by dual culture method was obtained with *Trichoderma harzianum* (38.89%). The next best antagonist in order of percent growth inhibition was *Bacillus subtilis* (32.64%). While in pathogen at centre method bacterial bioagents *Bacillus subtilis* (75.69%) was inhibitory than fungal bioagent *Trichoderma* sp.

Key words *Pythium, Isolation techniques, Soil, Bio-agent*

Chilli is a fruit of the plant '*Capsicum annum*' and '*Capsicum frutecens*' belonging to the family of 'Solanaceae'. *Capsicum annum* is a small in size, more pungent types and *Capsicum frutecens* is somewhat larger, mild to moderately pungent types. The pungency is due to the active principle capsicin contained in the skin and septa of the fruit, chilli are valued principally for their pungency and for their colour. Chilli forms an indispensable culinary spice in several parts of the world. It is also used in beverages and in the preparation of medicines. India is the second largest exporter of chilli in the world (Peter, 1999). In India, the crop is extensively cultivated in about 7.92 lakh hectares with a production of 11-12 lakh tones (Anon., 2011). Andhra Pradesh is the largest producer of chilli in India contributes about 27% to the total area under chilli followed by Karnataka (19%), Maharashtra (12%), Orissa (9%), Tamil Nadu (8%) and other states contributing 18% to the total area under chilli.(Anon., 2010)

Pythium aphanidermatum causes damping off disease (Mahmud 1952a ; Raghunathan 1968), root rot (Mahmud 1952b ; Ranghunathan and Marimuthu, 1973), foot rot (Singh 1998), rhizome rot (Kannan and Nair, 1965), and storage rot (Rao, 1966). Usually the fungus survives in soil in absence of suitable environment and host for infection. Among the various fungal diseases of chilli, damping-off caused by *Pythium aphanidermatum* (Edson) Fitzpatrick in nurseries is a major constraint in chilli production causing 62% mortality of seedlings (Ramamoorthy, *et al*., 2002). Rajagopalan, 1961 reported that *P. aphanidermatum* was the major species causing 75-80 per cent damping-off in tomato and chilli. Manoranjitham, *et al*., 2001 also reported 60 % mortality of chilli seedlings both in nursery and main field. The most common mean to check the disease caused by *P. aphanidermatum* in plants is by using fungicides. Frequent use of these chemicals leads to environmental pollution. The increasing awareness of fungicide-related hazards has emphasized the need of adopting biological methods as an alternative disease control method.

In view of above, isolation of the fungus from soil and efficacy of bio-agent is an important aspect for development of suitable management strategies. In the present study attempts have been made to develop suitable technique for isolation of *Pythium aphanidermatum* from soil and efficacy of bio-agents *in vitro*.

MATERIALS AND METHODS

Isolation technique was followed as suggested by Saha, *et al*., 2002 French bean was used as bait which stimulate growth of the pathogen. The fruit was dipped in a solution containing carbendazim (500 ppm) and streptomycin (100 ppm) for 12 hrs. for to make the fruit succulent and prevent contamination from bacteria and other fungi. The treated fruit was transversely cut into 2-4 pieces and buried into infested soil at a depth of 5-6 cm below the soil surface. The soil was irrigated and kept moist. After 24 to 48 hours, the bait fruits were removed from the soil in such a way that minimum disturbance should be to the pathogen invaded French bean/bottle gourd fruits. The fruits were placed inside air filled plastic bag individually under aseptic condition and kept at room temperature for 24 hours and by that time white fluffy mycelia growth were noticed on fruits, was aseptically transferred. The mycelial growth was observed under microscope and the culture was purified and maintained for further studies.

A total of eight bio-agents *viz*., *Trichoderma harzianum, T. viride, T. virens Pseudomonas fluorescens, Bacillus subtilis*, *Metarrhizium anisopliae, Paecilomyces*

lilacinus, Verticillium lecanii were screened for their effectiveness against *P. aphanidermatum* by two method *viz.,* dual culture method and Pathogen at centre techniques. In dual culture techniques (Kumar and Hooda, 2007) disc of six mm cut from the margins of actively growing cultures of antagonists and pathogens were placed at opposite points in petriplates 40 mm apart while in pathogen at centre method 6mm disc of the pathogen and four disc of the antagonist were placed equidistantly in the same petriplate simultaneously. Medium used was PDA and for each treatment four petriplates were maintained at 28±1. Side by side control plates were maintained for pathogen. The colony interaction were assayed as per cent growth inhibition of the radial growth of pathogen by formula- G_1-G_2/G_1 X 100 where G1 denote diameter of the radial growth of the pathogen in control plate and G2 denotes the radial growth of the pathogen towards the opponent antagonist in treated plate.

RESULTS AND DISCUSSION

Isolation of *Pythium* spices was carried out from infested nursery soil by using French bean/bottle guard as bait. Fruits were treated with carbendazin (500ppm) + streptocyclin (100ppm) solution for 24 hours and then it was transferred to infested soil. The entire french bean/ bottle guard fruits were covered with white fluffy mycelial growth within 24 hours and it was aseptically transferred to potato dextrose agar plates. Thus, the pathogen was isolated within five days without any other fungal and bacterial contamination.

The pathogen *Pythium* species was successfully isolated from the soil using french bean/ bottle guard fruit as bait, which is in conformity with the isolation technique suggested by Saha, *et al*., 2002. *Pythium* sp. have been isolated from soil by using carnation petals, cucumber seeds, pointed gourd and bottle gourd fruit as bait by earlier workers (Chamswarng, *et al*., 1991; Sinobas, *et al*., 1999) which are also in line with the present study. Saha, *et al*., 2002 isolated *Pythium aphanidermatum* from the soil by using pointed gourd as bait without any pretreatment while in the study french bean/bottle guard fruit was treated with carbandazim @ 500 ppm and streptomycin @ 100 ppm solution , as a result *Pythium* sp. could be isolated without any bacterial or fungal contamination in a comparatively shorter duration.

Dual Culture method:

The result (Table 1) indicated that all the antagonists significantly reduced the mycelial growth of the pathogen except *Trichoderma virens* and *Paecilomyces lillacinus*. Significantly maximum inhibition of mycelial growth of the pathogen after 72 hours of incubation was obtained with *Trichoderma harzianum* (38.89%). The next best antagonists in order of per cent growth inhibition were *Bacillus subtillis* (32.64%) followed by *Metarrhizium anisopliae* (29.86%), *Verticellium lecanii* (27.78%), *Trichoderma viride* (27.78%) which were at par with each other. Bacterial antagonist *Pseudomonas fluroscences* recorded minimum inhibition.

Table 1. Efficacy of bio-agents against *P. aphanidermatum in vitro* by dual culture method.

Sr. No.	Treatment	Radial growth (mm)	Per cent growth Inhibition
1	*Trichoderma harzianum*	55	38.89
2	*Trichoderma viride*	65.63	27.08
3	*Trichoderma virens*	90	0.00
4	*Paecilomyces lillacinus*	90	0.00
5	*Metarrhizium anisopliae*	63.13	29.86
6	*Verticelium lecanii*	65	27.78
7	*Pseudomonas fluroscences*	76.88	14.58
8	*Bacillus substillis*	60.63	32.64
9	Control (Without bio-agent)	90.0	00.0
	S.Em. ±	1.22	
	CD at 5 %	3.57	
	CV %	3.45	

Pathogen at centre method:

The results presented in (Table 2) revealed that the significantly maximum inhibition of mycelial growth of the pathogen after 72 hours of incubation was obtained with *Bacillus subtillis* (75.69). The next best in order of merit were *T. harzianum* (68.89), *Metarrhizium anisopliae* (60.58), *Verticelium lecanii* (56.25), *Pseudomonas*

Table 2. Efficacy of bio-agents against *P. aphanidermatum in vitro* by pathogen at centre method

Sr. No.	Treatment	Radial growth (mm)	Per cent growth Inhibition
1	*Trichoderma harzianum*	32.50	68.89
2	*Trichoderma viride*	43.75	51.39
3	*Trichoderma virens*	90.00	0.00
4	*Paecilomyces lillacinus*	90.00	0.00
5	*Metarrhizium anisopliae*	35.50	60.58
6	*Verticelium lecanii*	39.38	56.25
7	*Pseudomonas fluroscences*	39.38	56.25
8	*Bacillus substillis*	21.88	75.69
9	Control (Without bio-agent)	90.00	00.00
S. Em. ±		0.796	
CD at 5 %		2.323	
CV %		3.245	

fluorescens (56.25) and *T. viride* (51.39). Fungal bioagents *T. virens and Paecilomyces lillacinus* were found ineffective in inhibition of the pathogen and were *at par* with each other.

Among the two bacterial antagonists *B. subtillis* proved most effective compared to *P. fluorescens* in inhibiting the growth of *P. aphanidermatum.*

In present findings bacterial bioagents *Bacillus subtilis* was more effective to inhibit *Pythium aphanidermatum.* Similar evidence was reported by Intana *et al*., 2008, They reported that antagonists *Bacillus* spp. inhibited mycelial growth of *P. aphanidermatum.* Yoshida, *et al*., 2001 also reported that *Bacillus* sp. produced a clear zone which were able to produced antibiotics, provided better efficacy to inhibit mycelial growth of plant pathogens.

Our results are in line with results of Sharma, *et al*., 2003 and Yadav and Joshi, 2012 who reported fungal antagonist *T. harzianum* exhibited more than 50% and 67.98% inhibition of mycelial growth of *P. aphanidermatum* respectively.

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