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Evaluation of chemical and bio-control agents for management of *Cedrus deodara* root rot caused by *Phytophthora cinnamomi*

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ABSTRACT: *Phytophthora cinnamomi* was identified as the cause for drying up of deodar (*Cedrus deodara*) in the North-Western Himalayas. 50mg/ml concentration of Ridomil MZ was observed to inhibit the complete growth of *P. cinnamomi in vitro.* The impact of Ridomil MZ application on soil fungi revealed decrease in their population after 15 days of application but regained almost the original status within 30 days suggesting no lethal impact to the friendly rhizosphere fungi. *Trichoderma* spp. were observed as the most effective antagonists against *P. cinnamomi* among the associated fungal flora of *C. deodara* rhizosphere. The volatile compounds of the tested antagonists inhibited the growth of pathogen to a lesser extent as compared to dual culture. The application of the Ridomil MZ was identified as one of the immediate measure to control *P. cinnamomi*.

Key words: *Cedrus deodara*, *Phytophthora cinnamomi*, antagonists, Ridomil MZ, Chail forest

The Himalayan region is rich in bio-diversity comprising of lush green coniferous and broad leaved tree species supporting wide range of important herbs, shrubs, micro-organisms and fauna species. It controls flow of water during rainy season and supply water during summer season to the most important agricultural plains of India. Himachal Pradesh is dominated by important conifer species namely *Pinus gerardiana*, *Abies pindrow*, *Picea smithiana*, *Cedrus deodara*, *Pinus wallichiana* and *Pinus roxburghii* and is famous as Pine State of the country. Little work has been done on the forest pathology in India. Hardly any technology is available in this sector to control the disease outbreaks.

A serious root rot disease of *C. deodara* caused by *P. cinnamomi* was detected during 1998 (Singh and Lakhanpal, 2000) for the first time in the outskirts of Shimla near Chail in District Solan of Himachal Pradesh which wiped out the complete patch of about 7-8 hectare of pure *C. deodara* forest and is continuously spreading. In successive studies the area of the infected forest was estimated about 26 hectare (Karthikeyan *et al.,* 2000). Drying, dead and decaying trees still stands (Fig1a) as the witness to the disease damage in the forest. The macroscopic and microscopic examinations of the roots of trees with visible symptoms of drying revealed that most of the roots are infected with fungus (Fig1b). The genus *Phytophthora* is a devastating pathogen existing in a wide range of ecological niches (Erwin and Ribeiro, 1996). *P. cinnamomi* was first described in 1922 causing stripe canker of Cinnamon in Sumatra. Since then it has been reported as a pathogen for root rot of many broad leaved and coniferous trees.

In the absence of studies related to control measures of disease of forest tree species, State Forest Department is finding it difficult to control such serious infections in the forests. Therefore, a specific study was conducted under controlled conditions to test and develop chemical and biological control measures to contain the growth and reproduction of pathogen which will serve the basis for further refinement of technology in the management of the forest diseases.

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MATERIALS AND METHODS

Isolation of pathogen

The pathogen, *P. cinnamomi* was isolated from the infected roots of *C. deodara* by fruit trap method (Campbell, 1949). For the confirmation of identity of pathogen, zoosporangia were produced in non-sterile soil leachate as described by Mehrlich (1935).

Effect of fungicides/chemicals on pathogen growth

To assess efficacy of chemicals under *in vitro* conditions on the growth and development of pathogen, poisoned food technique was used (Nene and Thapliyal 1979). The stock solution of two fungicides namely Ridomil MZ 72 WP (metalaxyl 8% + mancozeb 64% WP), and Bavistin (carbendazim 50% WP), and inorganic chemicals namely Calcium Carbonate ($CaCO_3$) and Calcium Sulphate ($CaSO_4$) was prepared and four concentrations *viz.* 10, 25, 50, 100mg/ml were used in three replicates. These different concentrations were added into potato dextrose agar (PDA) culture medium just before pouring in the petriplates. Per cent growth inhibition of the pathogen culture was calculated by the standard formula.

Effect of fungicide treatment on rhizosphere soil fungi

To evaluate the toxicity of the fungicides to soil fungal flora, soil samples were collected from the infected forest and the status of fungi was studied. Soil fungi were isolated by

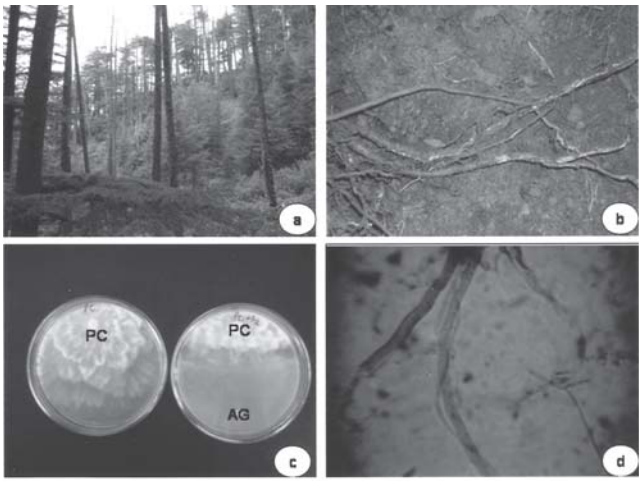

Fig. 1. (a) Diseased trees in forest (b) Infected roots (c) Growth inhibition in dual culture (PC - *P. cinnamomi*, AG - Antagonist *Trichoderma*) (d) Hyphal coiling

dilution plate method (Warcup, 1950). The effect of different dosages ranging from 1%-6% of the best selected fungicide (Ridomil MZ) were studied on the existing soil fungi population in the nursery bags. This was an important exercise because some of the fungicides are lethal to other friendly micro-flora of forest. The population of the fungal micro-flora was recorded from the date of application of fungicide at the interval of 15 days by soil dilution method.

Isolation and screening of antagonists

Soil samples were collected from the rhizosphere of healthy trees of different forest in the same locality and antagonists were isolated by the dilution plate method (Warcup, 1950). The pure cultures were maintained on PDA slants. Different rhizosphere fungi isolated from the rhizosphere of healthy trees were screened against *P. cinnamomi* by dual culture method (Dennis and Webster, 1971) on the PDA. Discs of 5mm cut from the margin of young growing cultures of antagonists and pathogen were placed at the opposite points in the plates 4cm apart from each other and incubated at 25±2°C. The growth of pathogen was recorded and compared with control to calculate the per cent growth inhibition.

Effect of volatile substances of antagonists

Pathogen and antagonists were inoculated in different petriplates on PDA. Petriplate after inoculation of the pathogen was immediately covered with inverted petriplate containing 4-5 days old colony of the antagonists and sealed with adhesive tape to assess the impact of volatile compounds of antagonists on pathogen. In control, the petriplate containing pathogen was inverted on petriplate without antagonist (Dennis and Webster, 1971). The growth of pathogen was recorded for next two days and growth inhibition of pathogen was calculated.

RESULTS AND DISCUSSION

Effect of fungicides/chemicals on the growth of pathogen

Among two fungicides and two inorganic chemicals tried in four different concentrations the statistical analysis of data revealed that *in vitro* growth inhibition of pathogen by Ridomil MZ was highly significant even at lower concentration (10mg/ml) as depicted in Table1. The higher concentrations further restrict the growth while in 50 and 100mg/ml no growth of pathogen was observed. Comparison among the two fungicides revealed that growth inhibition by Ridomil MZ was highly significant as compared to Bavistin and was selected for further experimentations. Growth inhibition by the $CaSO_4$ and $CaCO_3$ was significant only at higher concentrations ranging from 25-100 mg/ml (Table 1).

In an *in vitro* experiment 50mg/ml Ridomil MZ has been observed to completely inhibit the growth of *P. cinnamomi.* However, Bavistin has been found to slow down the growth (16.0%) of *P. cinnamomi* at 100 mg/ml concentration. There are several modes of action and arguments about controlling plant diseases through chemicals. Coffey and Joseph (1985) found that Phosphorus acid 4.1-6.2 mg/ml inhibited the mycelial growth of *P. cinnamomi in vitro.* Fenn and Coffey (1985) suggested that phosphate metabolism may be one target of phosphonate toxicity in Oomycetes. Similarly, the Ridomil MZ might have targeted the physiological process of the fungus to inhibit it under *in vitro* conditions.

Table 1. Effect of fungicides/chemicals on the mycelial growth of *Phytophthora cinnamomi*

Fungicides/	Mean diameter of mycelial growth in mm (% growth inhibition)				
Chemicals	10*	25*	50*	100*	Mean
Bavistin	63.7 (6.3)	63.0 (8.3)	62.3 (9.3)	57.7 (16.0)	61.7
Ridomil MZ	13.7 (80.0)	6.7 (90.3)	0.0 (100)	0.0 (100)	5.1
$CaSO_4$	68.0 (1.0)	67.3 (2.0)	66.7 (2.9)	65.3 (5.0)	66.1
$CaCO_3$	68.7 (0.0)	65.7 (4.4)	65.7 (4.4)	64.7 (5.8)	66.8
Mean	53.5	50.7	48.7	46.9	49.9
Control	68.7 (0.0)			G. mean	51.0
Factors	SED±	CD (P=0.05)			
Concentration	0.206	0.419			
Fungicides	0.206	0.419			
Treatments	0.412	0.838			

*Concentration of fungicides/chemicals in mg/ml

Effect of Ridomil MZ on the soil fungi

Studies on the effect of Ridomil MZ application on population of the rhizosphere fungi revealed a drastic reduction in mean percentage of population from 22.7% to 4.0 % after 15 days of its application but there was a good revival after 30 days of application (20.7%). After 30 days of application of the Ridomil MZ, the population of fungi recorded in concentration 1%-4% was nearly equal to the control while it was comparatively less in higher concentrations. The slight reduction of population observed in the control after 15 days might be due to change of soil from forest to nursery bags. Statistical analysis revealed that there was significant reduction in population of soil fungi in all treatment after the 15 days of application of Ridomil MZ (Table 2) and there was a significant regain of fungal population in soil after 30 days of Ridomil MZ application. Variation in population at 0 days is accounted for the application of different dosages in different containers/polybags in the nursery

It was observed that in general application of Ridomil MZ do not effect the fungal population for long and probable soil antagonist and mycorrhzial fungi were able to colonize in soil within 30 days of application of high concentration of Ridomil MZ.

Isolation and identification of antagonists

Total of 29 different fungi were isolated from rhizosphere of healthy *C. deodara* trees as fungal microflora. They were screened for the antagonism against pathogen and the fungi found antagonistic to the pathogen were processed for their taxonomical identification.

The growth of pathogen in dual culture was compared with control and per cent growth inhibition was calculated as explained under material and methods. Out of twenty-nine isolates, six isolates namely *Trichoderma harzianum, T. atroviride, Acremonium* sp*., Trichoderma* sp., *Pythium* sp. and *Trichoderma koningii* were antagonistic towards the pathogen. All of the antagonist form an inhibition zone with pathogen in dual culture on 3-4 day (Fig1c). The microscopic analysis of the hyphae at the zone of contact revealed the lysis and excessive vacuole formation in the pathogen hyphae. The coiling of antagonist hyphae around pathogen was also recorded in some cases (Fig1d). Maximum growth inhibition of 77.1%-77.6% of pathogen was recorded by two isolates of *Trichoderma* and minimum 59.5% by *T. koningii*. Statistical analysis of data revealed that the growth reduction of pathogen by antagonistic fungi was highly significant (Table3). Comparison among antagonists revealed the growth reduction was significant among themselves except *T. atroviride* and another isolate of *Trichoderma* sp. which appears to be the similar species/ strains. Pathogen growth inhibition by *Acremonium* sp. and *T. koningii* is at par with each other. Six fungi tested in dual culture for pathogen growth inhibition were identified and authenticated for their taxonomical identification from Forest Research Institute (FRI), Dehradun. Himachal Pradesh, Forest Department was intimated about the effectiveness of the chemical and biological control achieved *in vitro* to facilitate the studies under *in vivo* but no response was received.

Interactions between *P. cinnamomi* and antagonists (*Trichoderma*) involved coiling and parallel growth. Chambers and Scott (1995) also found that coiling, parallel growth and formation of apprisorium by *T. hamantum* and *T. pseudokoningii* inhibited growth of *P. cinnamomi*. There have been numerous reports of antagonisms of species of *Trichoderma* to pathogenic fungi including *P. cinnamomi* (Baker and Cook, 1974). On the contrary Kelley (1977) concluded that neither *T. harzianmum* nor *T. polysporum* was significantly antagonistic to *P. cinnamomi.* Aryantha and Guest (2006) observed antibiosis as the main mode of action although mycoparasitism, indicated by parallel hyphal growth, hyphal coiling, appresorium formation and direct penetration with one isolate of *Trichoderma* against *P. cinnamomi*.

Pythium sp. normally known for their pathogenic nature and casusing root rot of plants was found inhibiting growth of *P. cinnamomi* in significant proportion under dual culture conditions. *Pythium* infect a large range of hosts (Owen-Going, 2002), while *Phytophthora* spp. are generally more host-specific. Several *Pythium* species, including *P. oligandrum*, *P. nunn*, *P. periplocum*, and *P. acanthicum* are mycoparasites of plant pathogenic fungi and oomycetes, and have received interest as potential biocontrol agents. Similalry the presence of *Pythium* sp. alongwith *Phytophthora* in the *C. deodara* root rot and inhibition of *Phytophthora* growth in dual culture needs further studies to identify the exact role of *Pythium* sp. as antagonist or as a pathogen in *C. deodara* root rot.

The volatile compounds of all the tested antagonists inhibited the growth of pathogen to a lesser extent as compared to their performance in dual culture. The growth inhibition efficiency by the volatile compounds varied between 0.3%-10.4%.

Table 2. Effect of Ridomil MZ on the population of soil fungi in nursery bags

Incubation period (Days)	Number of colonies X 10^3 (Per cent population of microflora regained after application of Ridomil MZ)							
	0*	1*	2*	3*	4*	5*	6*	Mean
0	25.7	17.7	24.5	16.4	19.0	23.3	31.9	22.7
15	21.0	4.2	1.0	0.7	0.7	0.1	0.3	4.0
30	35.6	17.1	19.0	19.0	20.7	16.1	17.3	20.7
Mean	27.4	13.0	14.8	12.0	13.4	13.2	16.5	15.8

Factors SED± CD (P=0.05) Days 1.581, 3.192; Concentrations 2.415, 4.876; Treatments 4.183, 8.445
*Concentration of Ridomil MZ in per cent

Table 3. *Phytophthora cinnamomi* growth inhibition in dual culture

Fungal antagonist	Growth of pathogen (mm)	% Growth inhibition
P. cinnamomi + *Trichoderma harzianum*	18.7	72.7
P. cinnamomi + *Trichoderma atroviride*	15.3	77.6
P. cinnamomi + *Acremonium* sp.	27.3	60.0
P. cinnamomi + *Trichoderma* sp.	15.7	77.1
P. cinnamomi + *Pythium* sp.	20.3	70.3
P. cinnamomi + *Trichoderma koningii*	27.7	59.5
P. cinnamomi (Control)	68.3	0.0
G. mean	27.6	-
Factors	SED±	CD (P=0.05)
Treatments	0.563	1.209

The studies conducted and compiled is second stage efforts to develop control measures and threat abatement plan after identification of *C. deodara* root rot caused by *P. cinnamomi* by Singh and Lakhanpal (2000). Further studies are required to evaluate the potential of tested fungicide, inorganic chemicals, antagonists to determine the most effective, economical and environment friendly combination for application to control the disease in the infected forest. Nevertheless, the studies and results compiled here provide explanation for the potential of selected fungicide and antagonists for their practical application in the infected forest for the control of *C. deodara* root rot caused by *P. cinnamomi*.

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