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Assessment of Chemical and Biological Agents Against Important Forest Pathogens

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Abstract: Pathogens are known to cause losses to forest yield. Fungicidal application has been found to be effective against many pathogens but biological control holds promise as a strategy for disease management and it is environment friendly too. So, three pathogen having vast host range of forest plants viz., *Pestalotiopsis* sp., *Cerotocystis* sp., *Armillaria* sp. were selected for study. Approach includes *in vitro* efficacy of systemic and non-systemic chemical fungicides against selected pathogens along with *Trichoderma* species (*Trichoderma viride*, *T. harzianum*, *T. koningii*) as bio-control agent. Devithiram was found to be promising fungicide and *T. koningii* was best suitable as biocontrol.

Key words: Pathogen, fungicide, biocontrol.

Introduction

Pathogenic fungi are found widely distributed in moist deciduous, semi evergreen and evergreen forests and forest plantations. They show an enormous diversity in life-history strategies and the ways in which they interact with their hosts. These interactions range from species that form discrete lesions whose individual effects are very limited; to pathogens that establish perennial, systemic infection that castrate their hosts; and to pathogens that kill their hosts with considerable rapidity [3].

Many pathogenic fungi relies entirely on living host cells for sustenance and other can survive for a long period of time on dead host tissue or saprophytically in soil. Collectively, the pathogens can attack any plant part, although, individually they may be highly specialized. As a consequence, the range of pathogens found on different hosts also shows considerable diversity that may be associated with the evolutionary history of their hosts [4].

Forest plays an essential role in the life of mankind by providing food, timber, furniture and raw materials for all kinds of paper products. However, the productivity of forest generally is reduced when it becomes diseased. Pathogenic fungi invade the seeds in storage, nursery seedlings and forest trees subsequently resulting in low productivity and huge economic losses. Therefore, knowledge of forest pathogenic fungi in relation to forest health bears utmost importance especially with reference to their early detection and diagnosis followed by quick, timely remedial measures in order to avoid disease epidemics and timber losses. Therefore, this study focused on three highly destructive pathogens to the forest namely: *Pestalotiopsis* sp., *Ceratocystis* sp. and *Armillaria* sp.

Pestalotiopsis is a genus of ascomycete fungi which is soil borne pathogen that had been found to cause foliage and twig blight diseases. Two *Pestalotiopsis* species have been reported from Eucalyptus leaves, *P. disseminata* is reported to cause brown leaf blight on *Eucalyptus citridora* [12] while *P. funerea has* been reported to cause leaf spot on *E. globules* [13].

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Ceratocystis is a genus of Ascomycete fungi residing in the family Ceratocystidaceae (Order: Microascales~ Class: Sordariomycetes). Species of *Ceratocystis* are best known as wound infecting fungi. There are several group of species of this genera which are known to cause diseases in a variety of plants. *C. coerulescens* group causes blue stain in timber [6]. The majority of the species in the *C. fimbriata* group are primary pathogens causing cankers that can girdle and eventually kill the affected areas [15]. The third group of fungi in the broadly defined *C. moniliformis* group. This fungal species has also been reported from Dehradun for causing stem cankers in poplar plantations on the southern side of the trunk [9].

Armillaria (Agaricales, Physalacriaceae), species were first described by a Danish mycologist Martin Vahl in 1790, and in 1857 Fredrich Staude characterised the generic rank of *Armillaria* [11]. *A. mellea*, the most virulent of *Armillaria* spp. infects fruit, timber, and agronomic crops, as well as ornamental species and weeds [2]. Coetzee *et al.*.[5] reported *Armillaria* causing root rot of *Pinus wallichiana.*

Various approaches were investigated to manage forest pathogenic fungi causing seed deterioration, nursery diseases, foliar, stem and root diseases. In which, First of all and most useful is the application of the chemical fungicide to overcome the disease and its effects on to the host plants. These fungicides in various concentration in are found to be quite affective for variety of pathogens but continuous use of chemical fungicides may develop resistance in plant pathogenic fungi, therefore, alternative method must be followed along with the chemical fungicide for an effective disease control. One of such potential non-chemical alternative is the use of microorganisms as biological control agents for eco-friendly and sustainable management of plant disease. *Trichoderma* spp. are soil-borne fungi and have significant antagonistic potential against a wide range of phytopathogenic fungi. Furthermore, most of the *Trichoderma* spp. are known to produce volatile compounds such as acetaldehyde, ethylene, acetone and carbon dioxide and few produce antibiotics such as Trichodermin, gliotoxin, viridin and ergokonin accountable for antifungal and antibacterial properties. So, the objectives of the present work was *In-vitro* analysis of three important forest pathogenic fungi viz., *Pestalotiopsis* sp., *Cerotocystis* sp. and *Armillaria* sp. for their colony growth inhibition employing different measures such as biological and chemicals control experimentations involving chemical fungicides and microbial antagonists.

Materials and methods

The methodology for screening different chemical fungicides and microbial antagonists with respect to the selected fungal pathogens were finalized referring different techniques mentioned in the book by Dhingra and Sinclair [7]. The details of material and methods employed were as below:

Collection of forest pathogenic fungi

The cultures of forest pathogenic fungi *Armillaria* sp. (NTCC 1160) and *Cerotocystis* sp. (NTCC 1148) were obtained from National Type Culture Collection (N.T.C.C.) of Forest Pathology Division, Forest Research Institute (FRI) Dehradun. *Pestalotiopsis* sp. was isolated from *Eucalyptus* leaves which are collected from Uttarkhand State of North India.

Identification of pathogenic fungi

The identification of fungi was done based on the sporulation, morphology and colony characters of the fungus by referring to the 'Illustrated genera of Imperfect fungi'[1] and '*Dematiaceous hyphomycetes*' [8] for determining taxonomic identity of species. Thus obtained pure cultures were maintained on potato dextrose agar slants and stored in a refrigerator at 4^{o}C.

Evaluation of different fungicides for their effectiveness against forest pathogenic fungi

Four fungicides viz., Bayleton, Devithiram, Kavach Chlorothalonil and Devicopper (Table 1) were used to test the in vitro efficacy against three pathogenic fungi viz.,*Pestalotiopsis* sp., *Cerotocystis* sp., and *Armillaria* sp. by poisoned food technique and using Potato Dextrose Agar as basal culture medium. The required quantity of each fungicide was taken and thoroughly mixed

Table 1. Details of the fungicides

Mode of action	Fungicide	Chemical name	Active ingredient (Formulation)
Contact- Protective	Devicopper 50 WP	Copper Oxychloride	50% WP
	Kavach 75 WP	Chlorothalonil	75% WP
Systemic	Devithiram	Thiram	75% DS
	Bayleton 25 WP	Triadimef	25% WP

with autoclaved melted PDA medium to obtain required concentrations i.e., 0.25 %, 0.5 %, 1 % and 2 %. Twenty ml of poisoned melted PDA medium was poured into each sterilized plate and allowed to solidify. PDA medium without fungicides served as control. On solidification of PDA, all plates were inoculated by placing in the centre a 5 mm uniform mycelial disc of pathogenic fungi obtained from the eight days old cultures grown on PDA. Growth inhibition rate was recorded after 12 days of incubation. Observations on radial growth of the test pathogens were recorded against control plate. The bioefficacy of these fungicides was evaluated at different concentrations (ppm): 2500(0.25 %), 5000(0.5 %) 10,000(1 %) and 20,000(2 %) ppm.

Preparations of varying dosages of fungicides

Four fungicides were screened against some important pathogenic fungi. The required concentration or dosages were calculated by using following formula.

$$\frac{\text{Fungicides conc. to be tested} \times 1000}{\text{Active ingredient or effective concentration of fungicides}} = \text{amount of gms or ml per liter}$$

Screening of potential biocontrol agents by dual culture technique

Different fungal antagonists were evaluated against the pathogen through following method:

***In vitro* evaluation of bioagents**

The antagonistic potential of *Trichoderma viride, Trichoderma harzianum* and *Trichoderma koningii,* were evaluated for their effect *in-vitro* conditions against all pathogenic fungi by dual culture technique to screen the most efficacious one among them.

A) Calculation of percent growth inhibition by colony diameter

The colony diameter of both *Trichoderma* spp. and pathogens were measured at two locations, right angle to each other and the average diameter was calculated. Percent inhibition of mycelial growth of fungal pathogens over control was calculated by following formula given by Vincent [14]:

The percent growth inhibition was calculated by following formula:

$$I\ \% = [(C2-C1)/ C2)] \times 100$$

Where,

I = Per cent inhibition of mycelium

C2= Colony diameter in the control

C1 = Colony diameter in treated

The pathogen inhibition data were analyzed statistically in completely randomized design.

B) Calculation of percent growth inhibition by colony area

Antagonistic behaviour was measured quantitatively by calculating the area. Graph paper was used to measure the area of the antagonists, test pathogen species and inhibition zone in the petri plate. Antagonistic efficacy for each antagonist against the pathogen was worked out according to the following formula [10]:

$$\text{Antagonistic efficacy} = b + c - a$$

Where,

a = Percentage of area of test pathogen with antagonist in the same Petri plate (cm^2)

b = Percentage of area of antagonist, and

c = Percentage area of inhibition zone between antagonist and pathogen or overgrowth of antagonist over pathogenic fungi.

Results

Effect of different fungicides on colony growth of fungal pathogens

Laboratory evaluation of fungicides revealed that all the fungicides caused various inhibition of forest pathogenic fungi at various concentration (Table 2-4).

Response of *Pestalotiopsis* sp.to various concentrations of fungicides

Effect of fungicides against *Pestalotiopsis* sp.was observed in respect of radial growth. The results are presented in Table 2. Kavach 75 WP and Devithiram at low concentration (0.25 %) caused complete inhibition of mycelial growth followed by Bayleton 25 WP (0.23 cm) and Devicopper 50WP (2.5 cm). The present study illustrated the decrease in colony growth with the increase in concentration of fungicides.

Response of *Cerotocystis* sp.to various concentrations of fungicides

Four fungicides Devicopper 50WP, Kavach 75 WP, Devithiram and Bayleton 25WP at four concentrations each were tested against *Cerotocystis* sp. The results are accessible from Table 3. Among the four fungicides evaluated against *Cerotocystis* sp., Devicopper 50WP and Bayleton 25WP were found to be highly effective causing inhibition in mycelial growth of the fungus by all dosage of fungicides even in very low concentration of 0.25 %. Kavach 75 WP and Devithiram completely inhibit the radial growth of *Cerotocystis* sp. at 2 %. There is considerable difference observed in the suppression of fungus growth with each step up increase in concentration from 0.25 % to 2 % of medium.

Response of *Armillaria* sp.to various concentrations of fungicides

Four fungicides Devicopper 50WP, Kavach 75 WP, Devithiram and Bayleton 25WP at four concentrations each were tested against *Armillaria*

Table 2. Effect of different conc. of fungicides on the mycelial growth of the Pestalotiopsis sp.

Fungicide	Concentrations/ Radial growth of the pathogen in cm				
	Control	0.25 %	0.50 %	1 %	2 %
Devicopper 50WP	7*	2.5	1.33	1.33	0
Kavach 75 WP	7	0	0	0	0
Devithiram	7	0	0	0	0
Bayleton 25 WP	7	0.23	0.2	0	0

*Each value is the average of three replications

Table 3. Effect of different conc. of fungicides on themycelial growth of the Cerotocystis sp.

Fungicide	Concentrations/ Radial growth of the pathogen in cm				
	Control	0.25 %	0.50 %	1 %	2 %
Devicopper 50WP	7*	0	0	0	0
Kavach 75 WP	7	4.3	3.33	2.17	0
Devithiram	7	0	0.67	0.3	0
Bayleton 25 WP	7	0	0	0	0

*Each value is the average of three replications

Table 4. Effect of different conc. of fungicides on the mycelial growth of the Armillaria sp.

Fungicide	Concentrations/ Radial growth of the pathogen in cm Control	0.25 %	0.50 %	1 %	2 %
Devicopper 50WP	7*	2.3	1.1	0	0
Kavach 75 WP	7	1.8	0.7	0	0
Devithiram	7	0	0	0	0
Bayleton 25 WP	7	0	0	0	0

*Each value is the average of three replications

sp. The results are accessible from Table 4. Among the four fungicides evaluated against *Armillaria* sp., Devithiram and Bayleton 25WP were found to be highly effective causing inhibition in mycelial growth of the fungus by all dosage of fungicides even in very low concentration of 0.25 %. Devicopper 50WP and Kavach 75 WP completely inhibit the radial growth of *Armillaria* sp. at 1 % or more. There is considerable difference observed in the suppression of fungus growth with each step up increase in concentration from 0.25 % to 2 % of medium.

Biological control of pathogenic fungi

a) *Result of colony interaction as per percentage inhibition by colony diameter (cm)*

In dual culture plate assay the data was recorded on percent decrease in radial mycelial growth of different forest pathogens. The results revealed the significant effect on the radial mycelial growth inhibition of all pathogenic fungi by *Trichoderma* species as compared to control **(**Table 5).

Among the three antagonists, *T. koningii* was the most effective in inhibiting the mycelial growth of pathogenic fungi followed by *T. harzianum and T. viride*. Among the different pathogenic fungi, maximum percent inhibition of mycelial growth on average was observed in case of *Pestalotiopsis* sp. (27.68 %) followed by *Cerotocystis* sp. (24.35 %) and least inhibition of growth was observed in case of *Armillaria* sp. (5.07 %). Significant interaction showed that *T. koningii* inhibited the maximum growth of *Pestalotiopsis* sp**.** (39.47 %) among all the test pathogenic fungi (Plate No.1).

b) *Result of colony interaction as per percentage inhibition by colony area (cm^2)*

This method was calculated based on colony area of inhibition unlike measuring the diameter of the mycelial colony. In this method too result appeared similar proving *T. Koningii* (26.25 %) the highest potential as biocontrol. The method account antagonist efficacy so, in *Armillaria* sp.

Table 5. Antagonistic efficiency of *Trichoderma* spp. against Pathogenic fungi by colony diameter

No.	Pathogenic fungi	Mycelial inhibition % of pathogens by various *Trichoderms*spp. in dual culture *T. harzianum*	*T. viride*	*T. koningii*	Mean
1	Pestalotiopsis sp.	21.79*	21.79	39.47	27.68
2	Cerotocystis sp.	22.93	13.33	36.80	24.35
3	Armillaria sp.	11.50	3.70	0.00	5.07
	Mean	18.74	12.94	25.42	

*Each value is the average of three replications

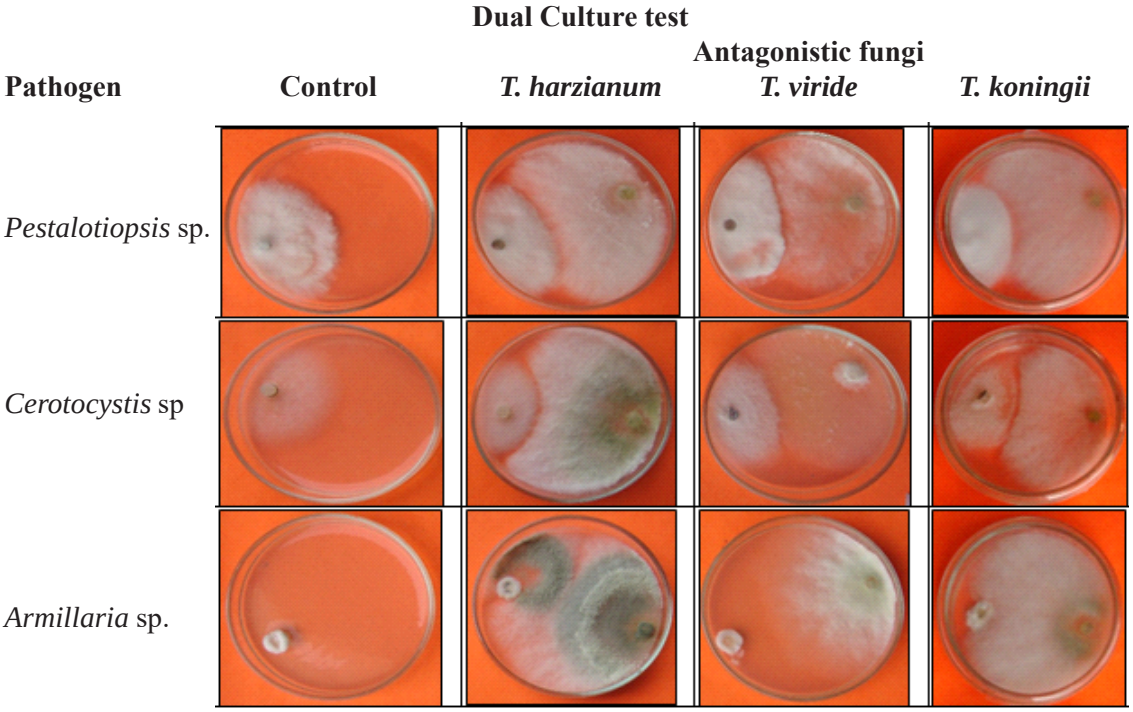

Plate 1. Dual Culture test of *Trichoderma* spp. against forest pathogenic fungi after five days of incubation

(35.27 %) was growth inhibition proving the highest inhibition. The minimum inhibition was noticed in *Pestalotiopsis* sp. (17.49 %). *T. harzianum* (26.16 %) was also found to be effective in inhibiting the growth of these forest pathogenic fungi whereas *T. viride* (18.61 %) showed the minimum antagonistic efficacy against the pathogens (Plate No. 1). Mycelium growth of *Pestalotiopsis* sp. was inhibited maximum by *T. koningii* (21.22 %) followed by *T. harzianum* (20.17 %) and the minimum by *T. viride* (11.09 %). Mycelium growth of *Cerotocystis* sp. was inhibited the maximum by *T. koningii* (24.85 %) followed by *T. harzianum* (23.58 %) and the minimum by *T. viride* (12.30 %). Mycelium growth of *Armillaria* sp. was inhibited the maximum by *T. viride* (38.45 %) followed by *T. harzianum* (34.74 %) and the minimum by *T. koningii* (32.61 %) (Table 6).

Discussion

From the present experiment, it is clear that in case of *Pestalotiopsis* sp. growth is completely inhibited by the fungicidal treatment of Kavach 75 WP and Devithiram even at very low concentration; *Armillaria* sp. Showed the complete inhibition by Devithiram and Bayleton 25 WP at

Table 6. Antagonistic efficiency of *Trichoderma* spp. by colony area

No.	Pathogenic fungi	Percent inhibition % of pathogens colony by various *Trichoderms*spp. in dual culture			
		T. harzianum	*T. viride*	*T. koningii*	Mean
1	Pestalotiopsis sp.	20.17*	11.09	21.22	17.49
2	Cerotocystis sp.	23.58	12.30	24.85	20.23
3	Armillaria sp.	34.74	38.45	32.61	35.27
	Mean	26.16	18.61	26.25	

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*Each value is the average of three replications

any concentration; and *Cerotocystis* sp. Showed complete inhibition at Devicopper 50 WP and Bayleton 25 WP. But overall, Devithiram was found to be most effective among the all fungicide as it completely inhibit the growth of the different pathogens at very low concentrations. On the other hand betweeb ecofriendly measures i.e. use of Trichoderma shows the different trend as among the all three species of Trichoderma, Trichoderma koningii found to be most promising as it shows the maximum suppression of the pathogens colony in the culture plates.

Furthermore, its effect on the growth of *Pestalotiopsis* sp. was significant. One more thing is interesting to be found that *T. Koningii* was found effective most but in case of *Armillaria* sp. the result were just reverse as it is affecting the growth least among all the *Trichoderma* sp. T. viride found least effective in controlling the growth of pathogens except *Armillaria sp.* where it was giving best results. *T. harzianum* showed the moderate results. It was neither most effective nor least effective. It is well known fact that chemical fungicide is best suitable for pathogen treatment yet it has some side effects too. But if we compare the effect of chemical fungicide with biocontrol agents in pathogen's growth suppression, we find that Biocontrol is better against low concentration of some chemical fungicide. But in broad sense chemical fungicide gives best results. Biocontrol can be effective in the field trials also if we uses the high doses i.e. we uses high spore concentration biocontrol media in the field but in that case, there might be a single chance of becoming of biocontrol agent to be pathogenic which needs to be find out.

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