

Original Research Article

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## Integrated Disease Management for Root Rot of Himalayan Fir (*Abies pindrow*) of Western Himalayas of Kashmir, India

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### ABSTRACT

Root rot is one of the major problems adversely effecting the growth of fir (*Abies pindrow*) seedlings in Kashmir valley and the study was conducted to find out the cause of the disease. The disease was prevalent in all the three fir growing nurseries surveyed with disease incidence 32.2 and 29.3 per cent during 2013 and 2014, respectively. The pathogens associated with the disease were isolated, morphologically characterized and identified as *Fusarium oxysporum* f.sp. *pini*, *Fusarium solani*, *Fusarium pallidoroseum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp. *Fusarium oxysporum* f.sp. *pini* was most predominant pathogen with isolation frequency of 30.0%, whereas *F. solani*, *F. pallidoroseum*, *R. solani*, *S. rolfsii* and *Pythium* sp showed isolation frequencies of 22, 18, 12, 10 and 8% respectively. All the fungi proved pathogenic and caused characteristic symptoms when inoculated separately. Among the various fungitoxicants evaluated against these pathogens during *in vitro* studies carbendazim and mancozeb exhibited maximum mycelial growth inhibition of 80.5 and 73.2 per cent, respectively. Various antagonists and ectomycorrhizal fungi evaluated through dual culture tests inhibited the growth of pathogens with varying degrees of inhibition. Among antagonists *Trichoderma harzianum* TG1 and *Trichoderma viride* TG2 and among the ectomycorrhizal fungi *Laccaria laccata* and *Boletus edulis* were most effective and showed mycoparasitic action and also developed zone of inhibition. During *in vivo* studies, fungitoxicants (carbendazim and mancozeb), antagonists (*T. harzianum* TG1 and *T. viride* TG2) and ectomycorrhizal fungi (*L. laccata* and *B. edulis*) used individually or in combination resulted in significant reduction in root rot intensity of fir seedlings. In comparison to control treatments resulted in a significant increase in growth parameters viz., shoot and root length and in turn increased the plant biomass. Bio-agents were at par with each other in respect of plant growth when evaluated individually. Bio-agents in combination showed synergistic growth promoting action and were superior in increasing shoot and root length of inoculated fir seedlings. Fungitoxicants individually or as component of treatment combination were significantly superior to bio-agents used singly or in combination. Among the fungitoxicants, carbendazim individually or in combination was significantly superior to mancozeb in reducing the disease intensity. Among the treatments, combination of *T. harzianum*, + *L. laccata* + carbendazim proved significantly superior to all the treatments with respect to reduction in disease intensity and increase in growth parameters and sturdiness quotient.

#### Keywords

Antagonists, Ectomycorrhizal fungi, Fir, Root rot pathogens and integrated disease management.

#### Article Info

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## Introduction

In India 76.95 million hectares are under forests with bearing 4,498.66 million m<sup>3</sup> total growing stock (Kiswan *et al.*, 2009). The estimated forest area in India under conifers alone is 0.045 million km<sup>2</sup> (Lal and Singh, 2003). As per the Forest Survey of India, forests cover in Jammu and Kashmir State is only 16,309 km<sup>2</sup>. Among these, conifers alone cover 40.87 per cent forest area (Anonymous, 2009). Conifers in Kashmir valley include *Pinus wallichiana*, *Pinus pindrosa*, *Abies pindrow*, *Cedrus deodara* and *Picea simthiana*. They are also planted in parks and large gardens for their aesthetic value. They provide wide range of socio-economic benefits through generation of employment, forest by-products and protection of sites of cultural value and are used as fuel, herbicide, lightening pitch, shelter-belt, stuffing and timber, and also for the extraction of turpentine, tar railway sleepers and packing crackers. Its seeds contain 28.7% oil which can be exploited commercially (Luna, 1996). The conifers are mainly propagated through nursery raised seedlings. Conifers are fairly strong timber and are easy to air season and kiln dry.

*Abies pindrow* (Family *Pinaceae*) commonly known as West Himalayan Fir, occurs in North Western Himalayas at an altitude of 3000-4500 m. Trees are 30 m tall or more, with a narrow pyramidal shape, bark fissured, light grey to brown, leaves spiral, 1-4 cm long, upper surface grooved, dark green and shiny. Male cones 1-2 cm long, axillary, ellipsoid, reddish-green; microsporophyll with two linear sporangia; microspores winged, female cones 8-12 cm long, solitary or in pairs, narrowly oblong, violetpurple; megasporophyll obovate, 2 cm long. Seeds 1-1.2 cm long; wing twice as long as the seed. These conifers are mainly propagated through nursery raised seedlings.

As in other conifers, fir is also threatened by many root rot causing pathogens and is prone to a number of diseases both at nursery as well as at plantation level posing a serious threat to their regeneration. Root rot problem in higher plantation is usually due to infestation of *Armillaria melleae* and few *Fomes* species however, the nursery plantation is threatened by many soil borne pathogens viz., *Pythium*, *Rhizoctonia*, *Fusarium*, *Phytophthora* and *Macrophomina*. These pathogens cause extensive damage to nursery plantation most of the infected seedlings fail to survive or establish after their transplantation (Douce *et al.*, 2002). Pathogens invade terminal un-suberised roots of young seedlings, penetrate through epidermal cell wall and grow intercellularly. They decompose cell wall materials and persist by metabolising cell contents resulting in damping-off or root rot and ultimately kill the host (Garret, 1970). In view of the destructive nature of root rot pathogens, various chemical, cultural and biological strategies have been explored to reduce the effect of these pathogens (Sanjay and Kaushik, 2001; Linderman *et al.*, 2006). Exploitation of biological agents as an alternative or complement to chemical treatment in integrated disease management strategies is widely gaining interest. As antagonistic microbes through their interaction with various soil borne plant pathogens play a vital role in maintaining microbial equilibrium and serve as powerful agents for biological diseases control. Symbionts especially mycorrhizal fungi which form integral component of fir rhizosphere are essential for growth and development of tree seedlings in soils having low nutrient content (Taylor and Alexander, 2005). Mycorrhizal root system benefits the host by increasing its root capacity to absorb nutrients from soil and also protect them from pathogenic invasion than non-mycorrhizal ones (Marx, 1969).

## Materials and Methods

### Survey for status of root rot of Fir

Periodic survey for the assessment of root diseases of fir was conducted during 2013 and 2014 in the months of May, August and November in three major fir growing nurseries of Kashmir valley, viz., Seer (Anantnag) and Nangdandi (Anantnag) and Tangmarg (Baramulla). Eight plots of 1 x 2 m size were randomly selected in each fir nursery and root rot incidence and intensity assessed on germinated fir. Total number of seedlings and the number of seedlings infected were recorded and the percent disease incidence worked out by using the following formula:

Disease incidence (%) =

$$\frac{\text{No. of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Root rot intensity was calculated on the basis of percentage of lateral root area affected by adopting scale described by Juzwik and Rugg, (1996) with slight modifications which is as follows:

#### Class descriptions

- 0 = No evidence of necrotic tissues.
- 1 = Laterals with 1-20% necrotic root
- 2 = Laterals with 20-50% necrotic root
- 3 = Laterals with 50-75% necrotic root
- 4 = Laterals with more than 75% necrotic root or necrotic lesion in the whole primary root

Per cent disease intensity =

$$\frac{\sum (n \times v)}{N \times G} \times 100$$

Where,

$\Sigma$ =Summation

n=Number of diseased seedlings in each category

v =Numerical value of each category

N=Number of seedlings examined

G=Maximum grade value

### Isolation, characterization and identification of pathogenic fungi

The fir seedlings with typical root rot symptoms were attempted for the isolation of associated pathogens. 5 mm segments of infected lateral roots were cut from these selected lateral roots at the zone of advancing decay.

The bits were surface sterilized and aseptically transferred to petri-plates containing potato dextrose agar (PDA) medium and incubated at 25±2°C. The plates were observed regularly for mycelial growth. Outgrowing hyphal tips were subcultured to develop a purified culture (Dasgupta, 1988). The isolated fungi were studied for their colony characters, mycelial growth and pigmentation.

Morphological characterization of isolated fungi viz., shape, size and septation of mycelium and spores were recorded. The characteristics observed were compared with authentic descriptions of Nelson *et al.*, (1983) and Sneh *et al.*, (1998). Pathogenicity was established by following Koch's postulates. For determination of isolation frequencies of root rot pathogens, three segments per root system were selected from ten randomly selected plants of each site. The number of isolates of fungal pathogens isolated from sampled root segments was determined and mean isolation frequency rates were calculated as the number of isolates of selected fungus from 30 sampled root bits.

### **Isolation, characterization and identification of biocontrol agents**

Ectomycorrhizae were collected underneath the fir plantation from three fir dominated forests. For isolation and culture of fungus, a shallow cut 1-2 mm deep to the sporocarp across the middle of cap along one side of the stipe to expose interior tissue. The tissue bits of 2-5 mm size from the expose interior surface were cut, loosened and aseptically transferred to the petri-plates containing modified Melin-Norkarn's medium (MMN) Marx, 1969. Plates were incubated for 25 days at  $25\pm 2^{\circ}\text{C}$  and regularly monitored for any mycelial growth. The colonies developed were studied for mycelial growth, colour and pigmentation. Morphological characteristics of isolated fungi viz., shape, size and septation of spores were also recorded. The macrofungi were identification on the basis of shape, size, colour and ornamentation of sporocarp and spores. The characters depicted by each fungus were compared with authentic description given by Godbout and Fotin (1985) and Lakhanpal (1988).

Rhizospheric soil from fir dominant areas was collected and rhizospheric antagonistic fungi were isolated using dilution plate technique and cultured on PDA (Elad *et al.*, 1981). The antagonists were identified on the basis of their morpho-cultural characteristics and spore characters and compared with authentic descriptions (Bissett, 1991; Gams and Bissett, 1998; Samuels, 2006). Both mycorrhizal and rhizospheric isolates were screened for their bio-control potential against fir root rot pathogens.

### ***In vitro* screening of bio-control agents against pathogens**

The *in vitro* antagonism in dual culture between ectomycorrhizal and the root rot pathogens was studied on MMN agar

according to Marx (1969). Five mm disc of 20 days old mycelial mat was placed about 2 cm away from the edge of 90 mm petri-plate containing 25 ml MMN agar medium. Fifteen days later 5 mm mycelial disc of test pathogen was placed on the opposite side of the plate, 4 cm away from the mycelial margin of the symbiont. Observations with respect to zone of inhibition on growth of the pathogen in dual culture as well as in control plates were recorded ten days after incubation. Antagonistic activity of rhizospheric fungal isolates against the test pathogens was assessed by dual culture technique (Utkhade and Rahe, 1983). Five mm mycelial discs each of ten days old culture of test pathogens and bio-control agents were placed on the fresh PDA plates about 60 mm apart in 90 mm petri-plate and incubated at  $25\pm 2^{\circ}\text{C}$ . The petri-plates with pathogen disc only served as control. Three replications for each treatment were laid out in a completely randomized design. Observations with respect to zone of inhibition on growth of the pathogen in dual culture as well as in control plates were recorded 7 days after incubation. The per cent inhibition in mycelial growth of the pathogen over control was calculated using formula given by Vincent (1947). For mass multiplication, ectomycorrhizal fungi were mass multiplied on peat moss mixture as per the method given by Marx and Bryan (1975) and fungal bioagents were mass multiplied on wheat bran (Younis *et al.*, 2010).

### ***In vitro* evaluation of fungitoxicants against the pathogens**

*In vitro* evaluation of various fungitoxicants to check the colony growth of the isolated pathogens evaluated through poisoned food technique on potato dextrose agar (PDA) medium (Borum and Sinclair, 1968). The experiment was conducted in completely randomized design (CRD) with 9 treatments

and 3 replications. Five systemic fungitoxicants viz., hexaconazole 5 EC, triadimefan 25 WP, carbendazim, 50WP, thiophonate methyl 70 WP, metalaxyl 68 WP, were tested each at 0.010%, 0.015%, 0.020%, 0.025% and 0.030% and four non-systemic fungitoxicants viz., copper oxychloride 50 WP, mancozeb 75 WP, chlorothalonil 75 WP and captan 50 WP, were tested each at 0.10%, 0.15%, 0.20%, 0.25% and 0.30% on formulation basis. 25 ml of PDA medium amended with fungicides in 5 different concentrations in separate 100 ml flasks was poured in petri-plates. The PDA medium without fungitoxicants was kept as control. 5 mm fungal disc of each test pathogen was picked from there purified cultures and inoculated in the center of each petri plate. Three replicate plates were inoculated for each fungitoxicant concentration. The inoculated plates were incubated at  $25 \pm 2^\circ\text{C}$  and mean colony diameter was measured once the control petri-plates get full growth. The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follows:

$$\text{Mycelial growth inhibition (\%)} = \left[ \frac{(dc-dt)}{dc} \right] \times 100$$

Where dc = average diameter of fungal colony in control, and  
dt= average diameter of fungal colony in treatment group

### Preparation of sick soil

Soil collected from SKUAST-K, main campus, was mixed with sand and forest litter in the ratio of 2:1:1 (w/w). The mixture was then autoclaved at 1.05 kg per  $\text{cm}^2$  for one hour for three consecutive days. One kg of potting mixture was put into each polythene bag of 1.5 kg capacity. The mass multiplied pathogenic inoculum @ 10 g/kg of mixture was incorporated 25 days before seedling transfer to develop sick soil culture.

### Inoculation and seed sowing

The interaction studies between most frequently occurring pathogen (*Fusarium oxysporum* f.sp. *pini*), most effective antagonists, ectomycorrhizae and fungitoxicants two from each were conducted in a polyhouse wherein a temperature of  $25 \pm 2^\circ\text{C}$  was maintained. The antagonists were inoculated singly and in combination @ 10 g/kg ( $1 \times 10^{-6}$  cfu) of mixture 7 days before seedling transfer and mixed properly. Mass multiplied ectomycorrhizal inocula @ 10 g/kg were added simultaneously with seedling transfer. The fungitoxicants (systemic @ 0.1% and non-systemic @ 0.3%) were applied as soil drench to the potting mixture 15 days after seedling transfer.

Five pre-germinated seeds having approximately 3-5 mm long hypocotyls were transplanted to each polybag and after establishment only one seedling was maintained per bag. The observations with respect to disease intensity, shoot length, root length, fresh weight, dry weight and sturdiness quotient were recorded on 300<sup>th</sup> day after seedling transplantation (DAT). Dry biomass of seedlings was determined by drying the plants at  $60^\circ\text{C}$  for 48 hours in a hot air oven. The experiment was laid in a randomized block design with three replications for each treatment. Sturdiness quotient was recorded by employing the formula given by Jaenicke (1999) which is as follows:

$$\text{Sturdiness quotient} = \frac{\text{Seedling height (cm)}}{\text{Collar diameter (mm)}} \times 100$$

The data wherever desired was subjected to appropriate transformations prior to analysis as suggested by Gomez and Gomez (1984).

## Results and Discussion

To assess the status of root rot in fir (*Abies pindrow*) seedlings in Kashmir valley, three major fir growing nurseries Seer and Nangdandi (Anantnag) and Tangmarg (Baramulla) were surveyed during two consecutive years 2013 and 2014. The survey was conducted thrice in a year starting from May at two months interval and the data obtained is presented in tables 1 and 2. The mean disease incidence at the locations surveyed varied from 24.6 to 36.7 per cent with maximum disease at Tangmarg and minimum at Seer. The disease incidence was higher in the year 2014 (32.2%) than in 2013 (29.3%). During the year 2013 mean disease incidence varied from 23.6 to 35.1 per cent while in the year 2014 it varied from 25.8 to 38.3 per cent. The disease shows progressive increase with time. The trend was same in both the years with maximum disease incidence 42.5 and 45.5 per cent noticed in November in 2013 and 2014, respectively, recorded at Tangmarg and least incidence of 27.3 to 30.0 per cent at Seer. Disease incidence at three locations varied significantly.

However, in the month of November highest disease incidence 44.0 per cent was recorded at Tangmarg which was statistically higher than 36.5 and 28.6 per cent recorded in Nangdandi and Seer, respectively. Disease incidence in the month of August at Tangmarg was 36.0 per cent which was statistically high with disease incidence in month of August at Nangdandi (30.2%) and Seer (24.2%) but proved statistically at par with November month disease incidence at Nangdandi. Least disease incidence was noticed in the month of May at Seer was 21.2 per cent which was statistically less with disease incidence in the month of May at Nangdandi (26.5%). and Tangmarg 30.2 per cent. Disease incidence at Nangdandi in the

month of May was 26.5 per cent which was statistically at par with disease incidence in the month of August (24.2%) at Seer. This significant increase may be attributed to favorable climatic and disease development conditions that existed during the period. Since the data recorded from two meteorological stations representing the areas of surveyed nurseries indicating the higher precipitation and temperature during 2014 than recorded during 2013. During the year 2014 precipitation and temperature at Gulmarg (Baramulla) was 713.0 millimeters and 16.4°C and at Kokarnag (Anantnag) 692.8 millimeters, and 22.3°C in comparison to 2013 precipitation 493.0 millimeters, temperature 17.8°C and 530.3 millimeters and 23.3°C at Gulmarg (Baramulla) and Kokarnag (Anantnag), respectively. High precipitation and warm weather had favours the pathogens so the root rot disease shows progressive increase with time. Ahanger *et al.*, (2011) was also of this opinion that warm weather (25-35°C) favours the pathogen to induce losses and predispose the conifers to infection by the root rot pathogens.

Two years pooled data revealed that the mean disease intensity at the locations surveyed varied from 22.9 to 32.7 per cent with maximum disease intensity at Tangmarg and least at Seer. The disease intensity was significantly higher in the year 2014 (29.3%) as compared to (24.7%) recorded during 2013. Disease intensity at the locations surveyed varied from 20.4 to 30.5 per cent and 25.5 to 35.0 per cent in the year 2013 and 2014, respectively. Comparison of two year data revealed least disease intensity of 22.9 per cent was recorded at Seer which was significantly lower than 25.4 per cent at Nangdandi. Maximum disease intensity was recorded at Tangmarg (32.7%) which shows significant difference with Nangdandi and Seer. Vaartaja and Morgan (1981) while working on root rot diseases in conifer

seedlings observed that high moisture content in the substrate favours the development of root rot pathogens. Similarly, Menkis *et al.*, (2005) reported that dry sandy loam substrate contributed to the low incidence of root rot pathogens of conifer seedlings.

Isolations from diseased roots of fir seedlings, collected during survey, revealed the presence of various pathogens viz., *Fusarium oxysporum* f.sp. *pini*, *F. solani*, *F. pallidroseum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp. *Fusarium oxysporum* f.sp.*pini*, was the most frequently isolated fungus with maximum frequency of 30.0%, whereas *F. solani*, *F. pallidroseum*, *R. solani*, *S. rolfsii* and *Pythium* sp showed isolation frequencies of 22, 18, 12, 10 and 8% respectively (Table 3). Initial symptoms in *F. oxysporum* f.sp. *pini*, *F. solani* and *F. pallidroseum* inoculated seedlings developed after 45<sup>th</sup>, 47<sup>th</sup> and 52<sup>th</sup>, day whereas in *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp. after 55<sup>th</sup> and 50<sup>th</sup> day of seedling transplantation to sick soil, respectively (Table 4).

Seedlings showed wilting of shoots, retarded growth, yellowing of needles and shredding of roots. Roots had developed partial or total rot, ultimately leading to death of root system and finally the whole plant. Menkis *et al.*, (2005) recorded 44.6 per cent isolation frequency of *F. oxysporum* from colonized roots of *Pinus sylvestris* seedlings and 0.3 per cent isolation frequency of *R. solani* from the diseased roots of *Picea abies* seedlings from Uppsala Sweden.

Ahanger *et al.*, (2011) were of this opinion that *F. oxysporum* as the most abundant pathogenic fungus in diseased roots of blue pine seedlings with isolation frequency of 38.6% where as *Rhizoctonia solani* and *Macrophomina phaseolina* showed isolation frequencies of 11.0 and 3.3 per cent, respectively.

Eight dominant ectomycorrhizal sporocarps (*Boletus edulis*, *Boletus rhodoxanthus*, *Laccaria laccata*, *Laccaria bicolor*, *Suillus granulatus*, *Suillus placidus*, *Russula lutea* and *Russula emetica*) were collected, identified, their ectomycorrhizal nature was established by observing their mycelial mat connections with feeder root tips. Isolations of ectomycorrhizal fungi were made on modified Melin-Norkans (MMN) agar medium from the sporocarps. Ten *Trichoderma* isolates were identified and isolated on PDA medium from fir rhizosphere. All the bio-control agents (ectomycorrhizal and rhizospheric antagonists) were screened against the isolated root rot fungi. *Laccaria laccata*, *Boletus edulis*, *Russula lutea* and *Suillus placidus* also have previously been reported and identified by Beig *et al.*, (2011) from Kashmir forests.

*In vitro* interactions between rhizospheric antagonists, ectomycorrhizal fungi and root rot pathogens (*Fusarium oxysporum* f.sp. *pini*, *F. solani*, *F. pallidroseum*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp.) revealed that all the tested bio-agents including mycorrhizae inhibited the mycelial growth of pathogens with varied degrees of inhibition (Table 4). The mycelial growth inhibitions ranged from 12.0 to 76.4 per cent. The maximum inhibition (60.2 %) was exhibited by *Trichoderma harzianum* TG1 followed by *T. viride* TG2 with 58.3-75.8 per cent inhibition. *Trichoderma harzianum* TG1 and *T. viride* TG2 caused twisting around the pathogenic hyphae and ultimately leading to lysis of parasitized hyphae. Mycorrhizae depicted strong mycoparasitic activity and showed significant antagonistic activity against root rot pathogens. *Boletus edulis* and *Laccaria laccata* developed zone of inhibition against the pathogens and caused 55.0-65.2 to 52.4-62.5 per cent mycelial growth inhibition. The involvement of strong antibiosis due to production of volatile metabolites and

diffusible chemicals is suggested for the formation zone of inhibition. Duchesne *et al.*, (1987) were of this opinion that antagonistic action of *L. laccata* may be attributed to the release of substantial antibiotics and other antimicrobial metabolites against the root rot pathogens. The mycoparasitic activity of ectomycorrhizal fungi against root rot pathogen *R. solani* has earlier been suggested by Zhao

and Kuo (1988). Our findings are in agreement with Amin *et al.*, (2010) and Jeyaseelan *et al.*, (2012) who reported that the application of *Trichoderma* sp. effectively controls a large number of soil borne fungi including *S. rolfsii*, *R. solani*, *Pythium* sp. *F. solani*, *F. oxysporum* and *F. pallidoroseum* in vegetables.

**Table.1** Cumulative root rot incidence on fir seedlings in major fir growing nurseries of Kashmir valley during the year 2013 and 2014

District	Location	Month	Root rot incidence (%)		
			2013	2014	Mean
Anantnag	Nagdandi	May	25.5 (30.3)	27.5 (31.6)	26.5 (30.9) <sup>d</sup>
		August	28.4 (32.2)	32.0 (34.4)	30.2 (33.3) <sup>c</sup>
		November	34.5 (35.9)	38.5 (38.3)	36.5 (37.1) <sup>b</sup>
		Mean	29.4 (32.8) <sup>B1</sup>	32.6 (34.7) <sup>B</sup>	31.0 (33.7)
	Seer	May	20.0 (26.5)	22.4 (28.2)	21.2 (27.3) <sup>e</sup>
		August	23.5 (28.9)	25.0 (30.0)	24.2 (29.4) <sup>d</sup>
		November	27.3 (31.4)	30.0 (33.2)	28.6 (32.3) <sup>c</sup>
		Mean	23.6 (28.9) <sup>C1</sup>	25.8 (30.4) <sup>C</sup>	24.6 (29.6)
Baramulla	Tangmarg	May	28.4 (32.2)	32.2 (34.5)	30.2 (33.3) <sup>c</sup>
		August	34.5 (35.9)	37.5 (37.7)	36.0 (36.8) <sup>b</sup>
		November	42.5 (40.6)	45.5 (42.4)	44.0 (41.5) <sup>a</sup>
		Mean	35.1 (36.2) <sup>A1</sup>	38.3 (38.2) <sup>A</sup>	36.7 (37.2)
Overall mean			29.3 (32.6)	32.2 (34.4)	-
CD (p= 0.05)			2.5	1.9	2.2

Figures in parenthesis are arc sine transformed value; Values superscripted with same letter (s) are statistically identical

**Table.2** Root rot intensity on fir seedlings in major fir growing nurseries of Kashmir valley during the year 2013 and 2014

District	Location	Root rot intensity (%)		
		2013	2014	Mean
Anantnag	Nagdandi	23.3 (28.8)	27.5 (31.6)	25.4 (30.2) b
Anantnag	Seer	20.4 (26.8)	25.5 (30.3)	22.9 (28.8) b
Baramulla	Tangmarg	30.5 (33.5)	35.0 (36.2)	32.7 (34.6) a
Mean		24.7 (29.7)	29.3 (32.7)	27.0 (31.2)
CD (p= 0.05)		1.7	1.2	1.4

Figures in parenthesis are arc sine transformed value; Values superscripted with same letter (s) are statistically identical



**Table.3** Isolation frequency (%) of root rot fungi and time taken for symptom expression in fir seedlings

Pathogen	Frequency of isolation (%age)	Days taken for symptom expression
<i>Fusarium oxysporum</i> f.sp. <i>pini</i>	30	45
<i>Fusarium solani</i>	22	47
<i>Fusarium pallidoroseum</i>	18	52
<i>Rhizoctonia solani</i>	12	55
<i>Sclerotium rolfsii</i>	10	55
<i>Pythium</i> sp	8	50

**Table.4** Mycelial growth inhibition (%) and zone of inhibition of fir root rot pathogens caused by potential bio-agents

Fungal/mycorrhizal species	Root rot pathogens					
	<i>Fusarium oxysporum</i>	<i>Fusarium pallidoroseum</i>	<i>Fusarium solani</i>	<i>Sclerotium rolfsii</i>	<i>Rhizoctonia solani</i>	<i>Pythium</i> sp.
<i>Trichoderma harzianum</i> TG <sub>1</sub>	76.4 (+)	73.0 (+)	70.5 (+)	72.0 (+)	74.8 (+)	60.2 (+)
<i>T. viride</i> TG <sub>2</sub>	75.8 (+)	72.0 (+)	68.3 (+)	68.6 (+)	73.9 (+)	58.3 (+)
<i>T. viride</i> TG <sub>3</sub>	74.6 (+)	71.5 (+)	65.2 (+)	68.0 (+)	70.3 (+)	55.5 (-)
<i>T. harzianum</i> TG <sub>4</sub>	70.0 (+)	67.5 (+)	63.3 (+)	64.6 (+)	70.1 (+)	52.3 (+)
<i>T. harzianum</i> TG <sub>5</sub>	74.0 (+)	65.3 (+)	60.1 (+)	67.3 (+)	45.3 (-)	53.2 (+)
<i>T. viride</i> TG <sub>6</sub>	73.5 (+)	54.8 (+)	45.8 (-)	61.2 (+)	69.7 (+)	45.8 (-)
<i>T. viride</i> TG <sub>7</sub>	40.2 (-)	36.2 (-)	33.3 (-)	18.0 (-)	16.1 (-)	24.4 (-)
<i>T. harzianum</i> TG <sub>8</sub>	34.2 (-)	24.6 (-)	18.0 (-)	34.6 (-)	39.5 (-)	32.5 (-)
<i>T. harzianum</i> TG <sub>9</sub>	38.2 (-)	25.2 (-)	39.5 (-)	36.4 (-)	42.1 (-)	14.0 (-)
<i>T. viride</i> TG <sub>10</sub>	25.3 (-)	17.3 (-)	37.6 (-)	25.5 (-)	34.5 (-)	26.0 (-)
<i>Boletus edulis</i>	65.2 (+)	62.8 (+)	65.0 (+)	62.2 (+)	63.0 (+)	55.0 (-)
<i>B. rhodoxanthus</i>	49.0 (-)	65.0 (-)	64.5 (-)	56.2 (-)	40.0 (-)	42.2(-)
<i>Laccaria laccata</i>	62.5 (+)	60.6 (+)	63.0 (+)	60.8 (+)	60.0 (+)	52.4 (-)
<i>Laccaria bicolor</i>	51.5 (-)	31.0 (-)	33.3(-)	12.0 (-)	34.2 (-)	34.2 (-)
<i>Suillus granulatus</i>	36.2 (-)	33.3 (-)	21.3 (-)	15.3 (-)	12.1 (-)	21.3 (-)
<i>Suillus placidus</i>	31.2 (-)	21.3 (-)	15.2 (-)	36.2 (-)	37.2 (-)	31.5 (-)
<i>Russula lutea</i>	36.5 (-)	23.2 (-)	37.2 (-)	34.6 (-)	40.1 (-)	12.1 (-)
<i>Russula emetica</i>	21.3 (-)	15.3 (-)	34.6 (-)	23.8 (-)	31.5 (-)	25.1 (-)

\*The (+) indicates the presence of zone of inhibition while (-) indicates the absence of zone

**Table.5** *In vitro* efficacy of systemic fungitoxicants in inhabiting the mycelial growth of test pathogens

Fungitoxicants/ Concentrations	Mean mycelial growth inhibition (%) at different concentrations					
	0.010 %	0.015%	0.020 %	0.025 %	0.030 %	Mean
Thiophenate Methyl 70 WP	50.5 (45.2)	64.2 (52.4)	83.3 (65.8)	100.0 (90.0)	100.0 (90.0)	79.6 (68.6) <sup>b</sup>
Hexaconazole 5EC	42.5 (40.6)	54.5 (47.5)	72.4 (58.3)	85.5 (67.6)	100.0 (90.0)	70.9 (60.8) <sup>c</sup>
Triadimefon 25 WP	40.2 (39.3)	52.5 (46.4)	65.0 (53.7)	76.5 (61.0)	92.4 (73.9)	65.3 (54.8) <sup>d</sup>
Carbendazim 50 WP	55.6 (48.2)	68.3 (55.7)	86.0 (68.0)	100.0 (90.0)	100.0 (90.0)	81.9 (70.3) <sup>a</sup>
Mean	47.2 (43.3) <sup>E</sup>	59.8 (50.5) <sup>D</sup>	76.6 (61.4) <sup>C</sup>	90.5 (77.1) <sup>B</sup>	98.1 (85.9) <sup>A</sup>	-

Figures in parenthesis are arc sine transformed value; Values superscripted with same letter (s) are statistically identical

CD (p= 0.05)

Fungicide (A) 1.2

Concentration (B) 1.2

(A x B) 2.0

**Table.6** *In vitro* efficacy of non-systemic fungitoxicants in inhabiting the mycelial growth test pathogens

Fungitoxicants/ Concentrations	Mean mycelial growth inhibition (%) at different concentrations					
	0.10 %	0.15%	0.20 %	0.25 %	0.30 %	Mean
Captan 50 WP	30.2 (34.4)	42.5 (39.3)	53.3 (45.2)	65.7 (52.8)	80.0 (62.0)	54.3 (46.7) <sup>d</sup>
Mancozeb 75 WP	55.3 (46.8)	62.5 (51.0)	74.4 (57.0)	86.5 (65.0)	100.0 (90.0)	75.7 (61.9) <sup>a</sup>
Coperoxychloride 50WP	36.8 (37.3)	45.7 (42.5)	58.2 (49.7)	74.5 (59.6)	92.5 (74.1)	61.5 (52.6) <sup>c</sup>
Chlorothalonil 75 WP	40.2 (40.6)	52.5 (46.4)	63.3 (52.7)	75.5 (60.3)	90.0 (71.5)	64.3 (54.3) <sup>b</sup>
Mean	40.6 (39.7) <sup>E</sup>	50.8 (44.8) <sup>D</sup>	62.3 (51.1) <sup>C</sup>	75.5 (59.4) <sup>B</sup>	90.6 (74.4) <sup>A</sup>	-

Figures in parenthesis are arc sine transformed value; Values superscripted with same letter (s) are statistically identical

CD (p= 0.05)

Fungicide (A) 1.0

Concentration (B) 1.0

(A x B) 2.4

**Table.7** Influence of fungitoxicants, antagonists and ectomycorrhizae individually and in combination on disease intensity, growth parameters and sturdiness quotient of fir seedlings grown in *F. oxysporum* f.sp. *pini* sick soil on 300<sup>th</sup> days after seedling transplantation

Treatments	Disease intensity	Shoot length (cm)	Root length (cm)	Collar diameter (mm)	Sturdiness quotient (cm)	Fresh weight (mg)	Dry weight (mg)
<i>F. oxysporum</i> (control)	93.3	3.1	3.7	7	0.97	170	70
Carbendazim	35.5	8.7	9.3	13	1.38	500	210
Mancozeb	46.5	5.5	6.7	10	1.22	375	160
<i>Trichoderma harzianum</i>	56.0	4.0	5.1	8	1.13	270	125
<i>Trichoderma viride</i>	56.5	3.8	4.8	8	1.08	267	123
<i>Boletus edulis</i>	59.0	3.5	4.4	8	1.00	264	116
<i>Laccaria laccata</i>	58.4	3.7	4.6	8	1.04	266	118
<i>T. harzianum</i> + carbendazim	27.2	9.6	11.0	14	1.47	616	268
<i>T. viride</i> + carbendazim	27.8	9.4	10.8	14	1.44	612	266
<i>B. edulis</i> + carbendazim	29.8	9.0	10.3	14	1.39	608	263
<i>L. laccata</i> + carbendazim	29.2	9.2	10.6	14	1.41	610	265
<i>T. harzianum</i> + mancozeb	40.4	8.0	8.4	12	1.33	478	208
<i>T. viride</i> + mancozeb	41.5	7.8	8.1	12	1.30	476	205
<i>B. edulis</i> + mancozeb	42.8	7.2	7.6	12	1.22	472	200
<i>L. laccata</i> + mancozeb	42.2	7.4	7.8	12	1.25	474	203
<i>T. harzianum</i> + <i>B. edulis</i>	51.5	4.8	5.5	9	1.14	295	142
<i>T. harzianum</i> + <i>L. laccata</i>	50.2	4.9	5.8	9	1.19	297	145
<i>T. viride</i> + <i>B. edulis</i>	52.8	4.4	5.2	9	1.07	292	135
<i>T. viride</i> + <i>L. laccata</i>	52.0	4.5	5.3	9	1.09	293	138
<i>T. harzianum</i> + <i>B. edulis</i> + carbendazim	13.6	12.6	14.2	16	1.68	824	365
<i>T. harzianum</i> + <i>L. laccata</i> + carbendazim	13.2	13.0	14.6	16	1.72	825	368
<i>T. harzianum</i> + <i>B. edulis</i> + mancozeb	18.0	10.5	12.7	15	1.56	698	305
<i>T. harzianum</i> + <i>L. laccata</i> + mancozeb	17.3	10.7	13.1	15	1.59	700	307
<i>T. viride</i> + <i>B. edulis</i> + carbendazim	16.0	12.0	13.8	16	1.61	820	360
<i>T. viride</i> + <i>L. laccata</i> + carbendazim	14.8	12.5	14.0	16	1.65	822	363
<i>T. viride</i> + <i>B. edulis</i> + mancozeb	19.0	10.2	12.0	15	1.50	695	300
<i>T. viride</i> + <i>L. laccata</i> + mancozeb	18.4	10.3	12.5	15	1.54	696	303
CD (p= 0.05)	2.1	1.1	1.0	3.2	0.04	4.0	3.0

The studies on *in vitro* evaluation of fungitoxicants against the root rot pathogens (*Fusarium oxysporum* f.sp. *pini*, *F. solani*, *F. pallidoroseum*, *Rhizoctonia solani*, *Sclerotium*

*rolfsii* and *Pythium* sp.) through poison food technique. Mycelial growth inhibition of each pathogen at each concentration was recorded and the mean mycelial growth inhibition of all

the pathogens is presented in tables 5 and 6. The data presented in the tables 5 and 6 revealed that the systemic fungitoxicant carbendazim exhibited highest mycelial growth inhibition 100.0% at 0.03% and non-systemic fungitoxicant mancozeb exhibited highest mycelial growth inhibition 100.0 % at 0.30%. Mycelial growth inhibition increases with increase in fungitoxicant concentration. Complete inhibition of colony growth of *F. solani* was observed by using benlate and carbendazim @ 100 ppm in bottle gourd (Nasreen and Ghaffar, 2010). The results obtained are in agreement to Kumar *et al.*, (2011) reported that Carbendazim and Vitavax were found to be more effective, showing 100% inhibition of mycelial growth of *Rhizoctonia bataticola* at 50 ppm followed by Kitazin which showed 100% inhibition at 100 ppm. Thiram, Blitox and Captan were the least effective.

*In vivo* experiments were laid to evaluate the most effective bio-agents (*T. harzianum* TG1, *T. viride* TG2, *L. laccata* and *B. edulis*) and fungitoxicants (carbendazim and mancozeb) individually or in combination for the management of most frequently isolated root rot pathogen (*Fusarium oxysporum* f.sp. *pini*) manipulated sick soil. All the bio-agents (antagonists and mycorrhizae) and fungitoxicants singly or in combination, significantly reduced root rot intensity as well as increased root length, shoot length, fresh weight and dry weight in fir seedlings in comparison to control (*Fusarium* inoculation alone) (Table 7). The percentage of disease reduction varied among treatments however, treatment combinations of bio-agents and fungitoxicant showed better results than their individual performances. In their, individual capacity, fungitoxicants proved significantly superior over bio-agents in reducing the disease intensity and increasing biomass. Among all individual treatments minimum root rot intensity (35.3 %) and maximum

shoot length (8.7 cm), root length (9.3 cm), fresh weight (500 mg) and dry weight (210 mg) were recorded in carbendazim in comparison to control. Statistical analysis indicated that all the bio-agents with disease intensity ranging between 56.0-59.0 per cent were at par with each other. Among the treatments components used singly maximum disease reduction over control of 61.9 per cent was recorded in carbendazim and least of 36.7 per cent in *B. edulis*. Antagonists in rhizosphere compete with pathogen for host surface and nutrients as well as inhibit pathogenic growth through antibiosis and mycoparasitism (Howell, 2003). Morsay *et al.*, (2009) reported *T. viride* resulted in 64.4 per cent suppression of *Fusarium* infection in tomato plants and increased 73 per cent survival rates. (Sharma, 2011) while working on root rot of *Pisum sativum* recorded 83 per cent disease control by carbendazim in comparison to 50 per cent by *T. harzianum*.

Dual combination of treatment components proved statistically superior to their individual performances in reducing the disease intensity ranged between 27.2 to 52.8 per cent and increases shoot length (4.4-9.6 cm), root length (5.2-11.0 cm), fresh weight (292-616 mg) and dry weight (135-268 mg). Combinations of bio-agents with fungitoxicants proved statistically superior to the combinations of bio-agents itself. Carbendazim + bio-agents proved significantly effective in reducing disease intensity than mancozeb + bio-agents. Superiority of combined effects of bio-agents and fungitoxicants also has been reported by Suriachandraselvan and Seethuraman (2002) who opined that in comparison to mono-inoculations, carbendazim in combination with *T. viride* resulted in highest reduction in root rot mortality of blackgram. Similarly Shashi *et al.*, (2007) reported that inoculation of *T. harzianum* and *P. fluorescens* with carbendazim, copper oxychloride, mancozeb

and thiophenate - methyl significantly lowered the *Fusarium* root rot incidence in tomato seedlings.

Among the treatments evaluated, combinations of all the three components (antagonists, mycorrhizal fungi and fungitoxicants) proved highly effective in reducing the disease intensity. On 300<sup>th</sup> DAT disease intensity varied from 13.2 to 59.0 per cent exhibiting 36.7 to 85.8 per cent disease control with maximum in treatment combination of *T. harzianum* + *L. laccata* + carbendazim and minimum in individual treatment of *B. edulis*. The disease intensity in such combination-treatment was significantly less to the disease intensities observed in their individual or in dual treatment combinations. Least disease intensity of 13.2 per cent was recorded in treatment combination of *T. harzianum* + *L. laccata* + carbendazim which was statistically at par with 13.6 and 14.8 per cent observed in *T. harzianum* + *B. edulis* + carbendazim and *T. viride* + *L. laccata* + carbendazim, respectively. Disease intensity of 19.0 per cent in treatment combination of *T. viride* + *B. edulis* + mancozeb was at par with 17.3, 18.0 and 18.4 per cent recorded in *T. harzianum* + *B. edulis* + mancozeb, *T. harzianum* + *L. laccata* + mancozeb and *T. viride* + *L. laccata* + mancozeb treatments, respectively. The study revealed that the treatment combinations with carbendazim as one component showed lesser disease intensity (13.2 to 16.0 per cent) and proved statistically superior over treatment-combinations involving mancozeb as fungitoxicants wherein the disease intensity ranged between 17.3 to 19.0 per cent.

The combined inoculation of biocontrol agents, ectomycorrhizal fungi and fungicides in *F. oxysporum* infested soil showed significantly better sturdiness quotient in comparison control. The maximum improvement (5.5%) in sturdiness quotient

was noticed in treatment where *T. harzianum* + *L. laccata* + carbendazim and minimum in *T. viride* + *B. edulis* + mancozeb (4.4%). *Trichoderma harzianum* + *L. laccata* + carbendazim were statistically superior to all the treatments except *T. harzianum* + *B. edulis* + carbendazim, *Trichoderma viride* + *L. laccata* + carbendazim, *T. viride* + *B. edulis* + carbendazim which were at par. *Trichoderma harzianum* + *L. laccata* + mancozeb were at par with *T. harzianum* + *B. edulis* + mancozeb, *T. viride* + *L. laccata* + mancozeb and *T. viride* + *B. edulis* + mancozeb. Bhan and Sharma (2011) reported that chemicals and seed stratification had increased the sturdiness quotient of apricot seedlings. Natalya *et al.*, (2011) were of this opinion that there was a drastic reduction in height and collar diameter of *Pongamia pinnata* seedlings due to various diseases. While working root rot of *Havea brasiliensis*, Ikerodah, *et al.*, (2012) observed that combined effect of *T. harzianum*, triadimefon or tridemorph resulted in linear infestation and increased growth.

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