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Fungi Associated with Root Rot of *Pinus wallichiana* Seedlings in Kashmir

[1]M.A. Ahangar, [1]G.H. Dar, [1]Z.A. Bhat and [2]N.R. Sofi
[1]Division of Plant Pathology,
[2]Division of Plant Breeding and Genetics,
S.K. University of Agricultural Sciences and Technology, Kashmir, 191121, India

Abstract: This study was conducted to find out the cause of root rot disease of blue pine (*Pinus wallichiana*) seedlings which is an important limiting factor in its successful production right from the nursery raising. The pathogens associated with the disease were isolated, morphologically characterised and identified as *Fusarium oxysporum* f.sp. *pini* (Schlecht.) Synd. and Hans., *Rhizoctonia solani* Kuhn and *Macrophomina phaseolina* (Tassi) Goid. *F. oxysporum* was the most abundant pathogenic fungus in diseased roots of blue pine seedlings with isolation frequency of 38.6%, whereas *R. solani* and *M. phaseolina* showed isolation frequencies of 11.0 and 3.3%, respectively. All the three fungi proved pathogenic and caused characteristic root rot symptoms when inoculated separately. Root rot disease of *Pinus wallichiana* seedlings was prevalent in all the three districts of Kashmir valley surveyed, during 2008 and 2009.

Key words: *Pinus wallichiana*, root rot, *Fusarium oxysporum, Rhizoctonia solani, Macrophomina phaseolina.*

INTRODUCTION

Pinus wallichiana (A.B. Jackson), the blue pine or Bhutan pine is an important conifer plant belongs to the family Pinaceae of order *Coniferales*. The blue pine is found in Himalayas at an elevation of 1500 to 3500 m a.m.s.l. In India the area under forests is estimated to be 639942 km^2, with 45234 km^2 under conifers alone. Of the total 20230 km^2 area under forests in Jammu and Kashmir, conifers cover an area of 40.87% out of which 9.73% is occupied by blue pine (Anonymous, 2008). Blue pine is propagated mainly through nursery-raised seedlings. Pines, like most of the forest trees, face severe problems in successful regeneration under existing natural conditions. Forest trees are often exposed to persistent pathogenic attacks, particularly those causing root rots. Among the conifers, root rot fungal pathogens have mostly been encountered on pines followed by deodar (Bhardwaj *et al*., 1988). The important root rot fungi posing serious threat to forest nurseries include the species of *Fusarium, Phytophthora, Pythium, Rhizoctonia, Macrophomina* and *Cylindrocladium* (Gupta *et al*., 1988; Huang and Kuhlman, 1990; Asiegbu *et al*., 1999). These pathogens often invade terminal unsuberized roots of young seedlings and cause late damping off or root rot thereby kill the host. The fungi penetrate into root epidermal cell wall, grow inter-cellularly, decompose cell wall materials and persist by metabolising cell contents (Garret, 1970). Root rot incidence/severity reduces the dry matter production and partitioning in affected plants (Aigbe and Remison, 2010). In present study *Fusarium oxysporum, Rhizoctonia solani* and *Macrophomina phaseolina* were found responsible for the root rot of *Pinus wallichiana* seedlings under temperate agroclimatic conditions of Kashmir. *Fusarium spp*. are well known forest nursery pathogens causing root rot in older seedlings as well as pre and post emergence damping-off, thus reducing seed germination and seedling survival (Machon *et al*., 2009). *F. Oxysporum* is the most important potential root pathogen in forest nurseries and is commonly associated with diseased conifer seedlings (Allin *et al*., 2004; Enebak *et al*., 1990). *Rhizoctonia* spp. are highly virulent pathogens of forest nurseries that can cause damping-off and root rot in seedlings (Chakravarty and Mishra, 2007). *Macrophomina phaseolina* is one of the most destructive plant pathogens that is both root and soil inhabiting and has a wide host range affecting more than 500 plant species including pines (Kadliesko, 1994). To increase biodiversity and to reduce soil erosion risk, besides revitalizing the rural economy and provide employment, has resulted an increase in the production of forest nursery seedlings. The early development of out planted seedlings in the field depends to a large extent on their physiological condition in the nursery. As such production of healthy seedlings is one of the main prerequisites for successful reforestation.

Correspondinng Author: M.A. Ahangar, Division of Plant Pathology, Division of Plant Breeding and Genetics, S.K. University of Agricultural Sciences and Technology, Kashmir, 191121, India

MATERIALS AND METHODS

During April-May 2008 and 2009, nine pine growing nurseries were surveyed from the three districts viz., Anantnag, Srinagar and Baramulla of Kashmir valley to assess the status of the disease. Three major nurseries were selected in each district. In each nursery six plots of 1×2 m size each were randomly selected, to assess the root rot incidence in one year old blue pine seedlings. Total number of seedlings examined and the number of seedlings showing root rot symptoms were recorded from each plot and percent disease incidence calculated by using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants observed}} \times 100$$

Ten plants from each surveyed nursery showing typical root rot/wilt symptoms were taken along with soil, bagged separately in polythene envelopes and immediately brought to the laboratory. The seedlings were then carefully uprooted and observations on deviated morphology of plant parts recorded. The seedlings with needle discolouration and stunted growth, following excavation also showed symptoms of root rot. The infected root portions were washed in running tap water. From each root system three to five main lateral roots showing decay symptoms were selected randomly. From each selected root, single segment of 5 mm in length was cut at the zone of advancing decay. The bits were surface sterilized in 0.1% mercuric chloride for one minute followed by three consecutive rinses in sterilized deionised water. The bits were blotter dried and then aseptically transferred to Potato Dextrose Agar (PDA) medium in sterile Petri-plates and incubated at 25±2°C. The plates were observed regularly and outgrowing mycelia were sub-cultured immediately. The cultures were purified by hyphal tip method (Dasgupta, 1988). Various cultural and morphological characteristics of isolated fungi were recorded by making visual and microscopic observations and compared with standard descriptions given by Nelson *et al*. (1983) and Sneh *et al*. (1991). The pathogenicity tests of root rot fungi isolated from the roots of diseased blue pine seedlings were carried out separately by following the Koch's postulate (Brock, 2000).

For the 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 the mean isolation frequency rates were calculated as the number of isolates of selected fungus from 30 sampled root bits.

RESULTS

To find out the status of root rot disease in blue pine (*Pinus wallichiana*) seedlings in Kashmir valley, various forest nurseries were surveyed during two consecutive years (2008 and 2009) in the month of May. The data presented in Table 1 revealed that mean disease incidence at the locations surveyed varied from 18.8 to 44.7% with overall mean incidence of 30.8%. Comparison of year-wise data revealed more disease prevalence (32.3%) during 2009 as compared to 2008 (29.4%). The degree of disease incidence varied from 17.2 to 43.2% and 20.4 to 46.2% in the year 2008 and 2009, respectively.

Isolations made from diseased roots of blue pine seedlings, collected during survey yielded three root rot fungal pathogens including *Fusarium oxysporum* f.sp. *pini* Schlecht., *Rhizoctonia solani* Kuhn and *Macrophomina phaseolina* (Tassi) Goid. Besides these, some saprophytic fungi viz., *Mucor, Rhizophus, Penicillium, Aspergillus* and *Trichoderma* spp. were also observed on affected root portions. The data on the isolation frequencies of root rot fungi revealed that *F. oxysporum* was isolated from all the surveyed nurseries with highest isolation frequency (38.6%), followed by

Table 1: Root rot incidence of Blue pine seedlings in various districts of Kashmir valley

		Root rot incidence (%)		
District	Location	2008	2009	Mean
Anantnag	Seer	17.2 (31.4)	20.4 (26.8)	18.8 (29.1)
	Ketriteng	43.2 (41.0)	46.2 (42.8)	44.7 (41.9)
	Achabal	27.5 (31.6)	32.2 (34.6)	29.9 (33.1)
	Mean	29.3 (34.6)	32.9 (34.7)	31.1 (34.6)
Srinagar	Lawipora	31.5 (34.1)	35.8 (36.7)	33.6 (35.4)
	Sonawar	38.6 (38.4)	41.4 (40.0)	40.0 (39.2)
	SKUAST-K, Shalimar	29.0 (32.5)	33.5 (34.2)	31.2 (33.5)
	Mean	33.0 (35.0)	36.9 (37.0)	34.9 (36.0)
Baramulla	Tangmarg	20.3 (26.7)	22.4 (28.2)	21.4 (27.4)
	J. V. Division Baramulla	32.5 (34.7)	31.4 (34.0)	31.9 (34.3)
	FoA Wadoora	24.1 (29.4)	28.5 (32.2)	26.3 (30.7)
	Mean	25.6 (3.02)	27.4 (31.4)	26.5 (30.8)
	Overall mean	29.4 (33.2)	32.3 (34.3)	30.8 (33.7)
	CD (p = 0.05)	1.02	0.96	0.94

Values in parenthesis are arc sine transformed values

Table 2: Isolation frequency (%) of root rot fungi from roots of blue pine seedlings

		Isolation frequency (%)*								
		Fusarium oxysporum			*Rhizoctonia solani*			*Macrophomina phaseolina*		
District	Location	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean
Anantnag	Seer	26.6* (31.0)	30.0 (33.2)	28.3 (32.1)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	3.3 (10.4)	1.6 (7.2)
	Ketriteng	46.6 (43.0)	53.3 (46.9)	49.9 (44.9)	13.3 (21.0)	16.6 (24.0)	15.0 (22.5)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
	Achabal	40.0 (39.2)	33.3 (35.2)	36.6 (37. 2)	10.0 (18.0)	0.0 (4.0)	5.0 (11.0)	10.0 (18.4)	6.6 (14.8)	8.3 (16.6)
	Mean	37.7 (37.7)	38.8 (38.5)	38.2 (38.1)	7.7 (14.3)	5.5 (10.6)	6.6 (12.4)	3.3 (9.7)	3.3 (9.7)	3.3 (9.7)
Srinagar	Lawipora	43.0 (40.9)	46.0 (42.7)	44.5 (41.8)	6.6 (14.8)	20.0 (26.5)	13.3 (20.6)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
	Sonawar	50.0 (45.0)	53.3 (46.9)	51.6 (49.1)	16.6 (24.0)	26.6 (31.1)	21.6 927.5)	6.6 (14.8)	3.3 (10.4)	5.0 (12.6)
	Shalimar	46.0 (42.7)	30.0 (33.2)	38.0 (37.9)	23.3 (21.0)	16.6 (24.2)	15.0 (22.6)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
	Mean	46.3 (42.8)	43.1 (41.9)	44.7 (42.3)	12.7 (19.9)	21.0 (27.2)	16.8 (24.5)	2.2 (7.6)	1.1 (6.1)	1.6 (6.8)
Baramulla	Tangmarg	26.6 (31.0)	36.6 (37.2)	31.6 (34.1)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)	3.3 (10.4)	0.0 (4.0)	1.6 (7.2)
	J.V. Division	43.3 (40.9)	33.3 (35.2)	38.3 (38.0)	13.3 (21.3)	20.0 (26.5)	16.6 (24.0)	10.0 (18.4)	6.6 (14.8)	8.3 (16.6)
	FoA Wadoora	33.3 (35.2)	26.6 (31.0)	30.0 (33.1)	10.0 (18.4)	16.6 (24.0)	13.3 (21.2)	0.0 (4.0)	0.0 (4.0)	0.0 (4.0)
	Mean	34.3 (35.4)	32.1 (34.5)	33.2 (35.0)	7.7 (14.5)	12.0 (18.1)	98. (16.3)	4.4 (10.9)	2.2 (7.6)	3.3 (9.2)
	Overall mean	39.3 (38.6)	38.0 (38.3)	38.6 (38.4)	9.3 (16.2)	12.8 (18.6)	11.0 (17.4)	3.3 (9.4)	3.3 (7.8)	3.3 (8.6)
	CD (p = 0.05)	6.5	7.3	6.0	4.1	7.6	6.7	1.0	4.3	3.0

*Percentage of colonies observed from 30 sampled root bits. Values in parenthesis are arc sine transformed values

R. solani (11.0%). *M. phaseolina* was occasionally isolated from sampled root bits with isolation frequency of 3.3% (Table 2).

DISCUSSION

Root rot disease caused by soil borne fungi is a serious problem faced by nursery growers throughout the world. Assessment of the status of a disease is one of the pre-requisites for devising suitable disease management strategies. The survey of forest nurseries of Kashmir valley revealed that root rot in blue pine seedlings was more or less prevalent in all the locations surveyed. The disease incidence varied from location to location. The disease incidence was significantly higher in the year 2009 which may be attributed to more precipitation (84.61 mm) and high temperature (23.53°C) prevailing during 2009 in comparison to 2008 (65.00 mm and 15.74°C, respectively). High moisture content (40-60%) in growth substrate generally favours the growth and infection by root rot pathogens of conifer seedlings. Bloomberg (1981) also reported that warm weather (25-35°C) favours the pathogen to induce losses and predispose the host to infection by the pathogen.

On the basis of morphological characters, pathogenicity and comparison with the authentic descriptions, the pathogens isolated were identified as *Fusarium oxysporum* f.sp. *pini* Schlecht. Synd and Hans., *Rhizoctonia solani* Kuhn. and *Macrophomina phaseolina* (Tassi) Goid. These pathogens have been reported elsewhere as causal organisms of root rot of conifer seedlings other than blue pine from Wisconsin (USA), Uppsala Sweden and Ontario forest nurseries (Bloomberg, 1981; Ocamb *et al*., 2002; Landis, 1999; Stepniewska-Jarosz *et al*., 2006; Pinto *et al*., 2006; Zakeri *et al*., 2010). The pathogenicity of isolated root rot fungi was established by proving Koch's postulates. The symptoms characterised to root rot were the needle discolouration, stunted growth and tip dieback. Root system showed lack of laterals and extensive cortical decay leading to the ultimate death of roots.

Observations recorded on isolation frequencies of root rot pathogens demonstrated that different fungi colonized the affected roots of blue pine seedlings in forest nurseries. The most frequently isolated fungal pathogens were *F. oxysporum, R. solani* and *M. phaseolina*. Some saprophytic fungi viz., *Rhizopus, Mucor, Penicillium, Aspergillus* and *Trichoderma* sp., were also isolated from diseased pine roots. *F. oxysporum* was the most frequently encountered pathogen in diseased roots of blue pine seedlings, with highest average isolation frequency of 38.6%. The isolation frequency of *R. solani* was 11.0% while as *M. phaseolina* was occasionally isolated with isolation frequency of 3.3%. These results corroborates the findings of Menkis *et al.* (2006), who recorded 44.6% isolation frequency of *F. oxysporum* from colonized roots of *Pinus sylvestris* seedlings and 0.3% isolation frequency of *R. solani* from the diseased roots of *Picea abies* seedlings from Uppsala Sweden. *Fusarium* root rot of forest nursery seedlings is most often attributed to the fungus *Fusarium oxysporum,* although some other Fusaria have sometimes been implicated in root rot of conifer nursery seedlings (Enebak *et al*., 1990; Juzwik and Rugg, 1996). Barbara and Leszek, (2004) observed that *R. solani* is responsible for damping-off and root rot in young pine seedlings. Barnard *et al*., (1994) also reported that *M. phaseolina* caused charcoal root rot and is a factor in the development of black root rot together with *Fusarium* spp. in some forest tree nurseries.

In conclusion root rot of blue pine seedlings was observed at moderate to high levels in all the nurseries surveyed and three most potential pathogens were found responsible, there by poses a serious threat to the regeneration of pine plantation. As such control measures that are devised for root rot must be taken into account in order to meet the demand of conifer nursery seedlings by producing more healthy seedlings.

REFERENCES

Aigbe, S.O. and S.U. Remison, 2010. The influence of root rot on dry matter partition of three cassava cultivars planted in different agro-ecological environments. Asian J. Plant Pathol., 4: 82-89.

Allin, T.W., S.A. Eneaek and W.A. Carey, 2004. Evaluation of fungicides for control of species of *Fusarium* on long leaf pine seed. Crop Protect., 23: 979-982.

Anonymous, 2008. A digest of forest statistics. Statistics Division, Department of Forests, Jammu and Kashmir Government, Srinagar India, pp: 15.

Asiegbu, F.O., M. Kacprzak, G. Daniel, M. Johansson, J. Stenlid and M. Manka, 1999. Biochemical interactions of conifer seedling roots with *Fusarium* spp. Can. J. Microbiol., 45: 923-935.

Barbara, D. and B.O. Leszek, 2004. *Rhizoctonia solani* on coniferous seedlings in forest nurseries. J. Plant Prot. Res., 44: 175-180.

Barnard, E.L., S.P. Gilly and E.C. Ash, 1994. An evaluation of dazomet and metam-sodium soil fumigants for control of *Macrophomina phaseolina* in a Florida forest nursery. Tree Planters Notes, 45: 91-95.

Bhardwaj, L.N., P.K. Seth and R.C. Sharma, 1988. Seed Mycoflora of Forest Tree Species–Their Role in Seedling Diseases and Management. In: Tree Protection, Gupta, V.K. and N.K. Sharma (Eds.). Indian Society of Tree Scientists, India.

Bloomberg, W.J., 1981. Disease Caused by *Fusarium* in Forest Nurseries, In: *Fusarium*: Diseases, Biology and Taxonomy, Nelson, P.E. (Ed.). Pennsylvania State University Press, USA.

Brock, T.D., 2000. Robert Koch: A Life in Medicine and Bacteriology. ASM Press, Washington, DC, USA., pp: 364.

Chakravarty, P. and R.R. Mishra, 2007. Studies in forest pathology root rot due to *Rhizoctonia solani* Kunn. Eur. J. Forest Pathol., 12: 308-316.

Dasgupta, M.K., 1988. Principles of Plant Pathology. Allied Publishers Pvt. Ltd., Banglore, pp: 1140.

Enebak, S.A., M.A. Palmer and R.A. Blanchette, 1990. Managing soilborne pathogens of white pine in a forest nursery. Plant Dis., 74: 195-198.

Garret, S.D., 1970. Pathogenic Root Infecting Fungi. Cambridge University Press, London, UK.

Gupta, V.K., R.C. Sharma, R.C. Garg and J.C. Kaushik, 1988. Forest Plant Diseases and their Management. In: Tree Protection, Gupta, V.K. and N.K. Sharma (Eds.). Indian Society of Tree Scientists, India.

Huang, J.W. and E.G. Kuhlman, 1990. Fungi associated with damping off of slash pine seedlings in Georgia. Plant Dis., 74: 27-30.

Juzwik, J. and D.J. Rugg, 1996. Seedling mortality and development of root rot in white pine seedling in two bare-root nurseries. Can. J. Plant Pathol., 18: 335-341.

Kadliesko, S., 1994. Some new results on the host range of *Macrophomina phaseolina* in hungary. Acta Phytopathol. Entomol. Hungarica, 29: 61-66.

Landis, T.T., 1999. Disease and Pest Management. In: The Container Tree Nursery Manual, Tinus, T.D., R.W. Mc-Donald, S.E. Barnett and J.P. Landis (Eds.). Department of Agriculture-Forest Service, USA.

Machon, P., J.A. Pajares, D.J. Diez and F.M. Alves-Santos, 2009. Influence of ectomycorrhizal fungus *Laccaria laccata* on pre-emergence, post-emergence and late damping-off by *Fusarium oxysporum* and *F. verticillioides* on stone pine seedlings. Symbiosis, 49: 101-109.

Menkis, A., R. Veslliauskas, A.F.S. Taylor, E. Stenstrom, J. Stenlid and R. Finlay, 2006. Fungi in decayed roots of conifer seedlings in forest nurseries, afforested clear-cuts and abandoned farmland. Plant Pathol., 55: 117-129.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium* Species: An Illustrated Manual for Identification. 1st Edn., Pennsylvania State University Press, University Park, Pennsylvania, ISBN-10: 0271003499.

Ocamb, C.M., J. Juzwik and F.B. Martin, 2002. *Fusarium* spp and *Pinus strobes* seedling: Root disease pathogens and taxa associated with seed. New Forests, 24: 67-79.

Pinto, P.M., J. A.P. Alonso, V.P. Fernandez and J.J.D. Casero, 2006. Fungi isolated from diseased nursery seedlings in Spain. New Forests, 31: 41-56.

Sneh, B., L. Burpee and A. Ogoshi, 1991. Identification of *Rhizoctonia* species. APS Press, St. Paul, MN.

Stepniewska-Jarosz, S., M. Manka and F.O. Aslegbu, 2006. Studies on anaestomosis groups of *Rhizoctonia solani* isolates causing disease in two forest nurseries in Poland. Forest Pathol., 35: 97-109.

Zakeri, A., H. Hamzeharghani, Z. Banihashemi and S.H. Saadati, 2010. Pathogenic fungi associated with pre- and post-emergence seedling blight of pine and cypress in Fars Province, Iran. Forest Pathol., (In Press).