Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

INCIDENCE OF CHIR PINE (*PINUS ROXBURGHII*) NURSERY DISEASES IN MURREE HILLS AND THEIR *IN VITRO* MANAGEMENT

[a]Aqeel Haider, [a]Abid Riaz, [a]Abdul sattar, [b]Muhammad Farooq
[a] Department of Plant Pathology, PMAS Arid Agriculture University, Rawalpindi, Pakistan.
[b] Forest Department, Murree Division, ,Government of the Punjab, Pakistan.

A B S T R A C T

In the present study, incidence of diseases of Chir pine plants grown in various nurseries located at Lakot, Ghora Gali and Tret (Murree) was determined during June, 2014 survey on the basis of symptoms and association of various fungi was confirmed by performing pathogenicity tests. The survey revealed that Chir pine (*Pinus roxburghii*) plants were suffering from post-emergence damping off (root rot) and needle blight. Highest disease incidence of root rot (15%) was found in Tret nursery and lowest at forest nursery, Ghora Gali (6%) while of needle blight was 9% and 13% at forest nursery and Forest Research Institute Faisalabad Nursery, respectively. Isolation and identification revealed the presence of four fungi viz., *Fusarium spp, Pestalotiopsis spp, Botryodiplodia spp* and *Rhizoctonia spp*. *Fusarium spp* and *Rhizoctonia spp* have been reported causing considerable economic losses to other agricultural plants in Pakistan but not reported from forest plants before. This study is first of its kind regarding determination of incidence of chir pine nursery diseases and their *in vitro* management and calls for adoption of some precautionary measures to manage the diseases especially damping off/root rot.

Keywords: Chir Pine, needle blight, nursery diseases, root rot.

INTRODUCTION

Pinus roxburghii (Chir pine) is considered to be an important species (Gupta and Dass, 2007) occurring at elevations from 900 to 1800 m above sea level (Siddiqui *et al.*, 2009). Numerous pathogens attack Chir seedlings and trees both in nurseries and forests. Damping-off is the most common disease that affects seed germination and young seedlings (Huang and Kuhlman, 1990). The disease is caused mainly by soil borne pathogenic fungi among which *Fusarium* species have been considered particularly damaging (Bloomberg, 1981 and Lori *et al.,* 1999). According to Jones and Averre (2000), species of *Rhizoctonia, Fusarium, Sclerotium rolfsii and Macrophomina phaseolina* cause damping-off under warmer and drier conditions and these have seriously limited the large scale production of these plants in the area.

Chir pine trees are attacked by number of other fungal diseases which cause abundant losses to the economy of the state. Among those are Diplodia Blight of Pines, Diplodia tip blight, Diplodia dieback, Diplodia shoot Blight (*Diplodia spp*), Pitch canker, Stem rot, root rot, yellowing and browning of needles (*Rhizoctonia spp*), Pestalotiopsis foliage blight, canker, dieback, needle disease, tip blights, grey blights and cholrosis,(*Pestalotiopsis spp*) (Sturrock *et al.*, 2011). Pitch canker, is the most damaging disease of pines in the entire world which is caused by *Fusarium circinatum* (Carlucci *et al.*, 2007).

Needles are attacked by variety of fungi which cause many diseases on them. The disease pattern and dissemination of shoots, foliage and crown provides valuable evidences about the attacking pathogens. Naturally shoot affecting fungi also cause infection on the needles and stems and cause injury in the lower crown of disease plants (Brown *et al.*, 2005). The present study was undertaken to determine the current status of Chir pine nursery diseases in Murree and subsequently *in vitro* management of the fungi responsible for those diseases. Therefore, with respect to the latter objective, inhibition of mycelial growth of

* Corresponding Author:
Email: abidriaz@uaar.edu.pk

four fungi exposed to different concentrations of various fungicides and *Polygonium* extract was investigated to know their most effective concentrations.

MATERIALS AND METHODS

Sample collection and disease incidence: Different nurseries of Murree were surveyed in 2014. Disease samples were collected from different nurseries located in Lakot, Ghora Gali, and Tret. The other purpose of survey was to observe the disease incidence which was calculated by following formula.

$$Disease\ Incidence\ (\%) = \frac{No.\ of\ Diseased\ Plants}{Total\ No.\ of Plants} \times 100$$

***In vitro* management of Chir Pine's pathogens**

Response of identified mycoflora to three different fungicides: Toxicity of three fungicides viz., Dithane M-45, Ridomil Gold, and Topsin-M were tested against four fungi viz., Fusarium spp, Pestalotiopsis spp, Botryodiplodia spp. and Rhizoctonia spp. by the poisoned food technique. Each fungicide was mixed separately in autoclaved melted PDA medium to obtain required concentrations i.e., 1%, 5%, 10%, 15% and 20%. Twenty ml of poisoned melted PDA medium was poured into each sterilized plate and allowed to solidify. PDA medium without fungicides served as control. After solidification of medium, 3 mm agar plugs of the fungus on PDA were transferred in the center of the plates. Each treatment was replicated thrice. All the plates were incubated at 25±2°C. Growth inhibition rate was recorded after 10 days of incubation.

Response of the identified mycoflora to Polygonium extract: *Polygonium amplexicaule* used in the experiment was processed following Singh *et al.*, (1997). Petals were collected from the flower garden of PMAS-AAUR. The fresh material of *Polygonium* was dried in shadow and then crushed in porcelain mortar. A weighed quantity of *Polygonium* was taken into flask to which 60 ml of methanol (Riedel Haen, Germany) was added. After adding the flask were kept at magnetic stirrer for 4 hour at room temperature. The supernatant was discarded. The solvent was vaporized by keeping the flask in fume hood approximately for 10 hour. Samples were washed with 10 ml of methanol. The extracted material was sterilized by passing through a Millipore filter paper. Extract was prepared at five different concentrations for each fungus. *Polygonium* extract was mixed separately in autoclaved melted PDA medium to obtain required concentration i.e., 1%, 5%, 10%, 15% and 20%. Inoculation, incubation and measurements of radial growth of the fungus were done using the same procedure as done in case of fungicides.

Statistical analysis: Mycelial growth inhibition was analyzed using MSTAT-C with 2 factor factorial Completely Randomized Design (CRD). Analysis of variance was used to test difference between treatments. Treatments means were separated by least significant difference (LSD).

RESULTS AND DISCUSSION

Disease incidence and sample collection: The % incidence of diseases observed from four nurseries one located at each Lakot and Tret and two at Ghora Gali on the basis of symptoms is shown in the Table-1. The results showed that the percentage incidence of diseases was varying at all nurseries.

Table 1. Disease incidence (%) on Chir pine plants at different localities of Murree region

Sr. #	Location	Disease Incidence		Fungi Associated
		Damping off / Root Rot	Needle Blight	
1	Forest Nursery (Ghora Gali)	6%	9%	*Rhizoctonia spp.,Pestalotiopsis spp., Botryodiplodia spp.*
2	Awain Forest Nursery (Lakot)	9%	-	*Fusarium spp. Rhizoctonia spp*
3	(FRIFN)* (Ghora Gali)	13%	13%	*Rhizoctonia spp; Pestalotiopsis spp. Botryodiplodia spp.*
4	Tret Forest nursery (Tret)	15%	-	*Rhizoctonia spp Fusarium spp.*

*FRIFN= Forest Research Institute Faisalabad Nursery.

The result indicates that highest disease incidence of root rot (15%) was found among chir pine in Tret nursery and lowest disease incidence of the above pathogen was found in Ghora Gali (6%). In case of needle blight the maximum number of blighted plants was found in Forest Research Institute Faisalabad, Nursery (FRIFN) with incidence of (13%) and (9%) and no incidence was recorded in Lakot and Tret. The mean incidence of diseases from all nurseries was found to be (11%) in each of root rot and needle blight which was alarming and needs immediate attention of the department to minimize the losses.

Response of *Pestalotiopsis Spp* to various concentrations of fungicides and polygonium extract: Effect of fungicides and *Polygonium* extract against *Pestalotiopsis spp* was significantly different from the control in respect of radial growth. The results are presented in Table 2. However, the Ridomil gold at highest concentration (20%) caused complete reduction of mycelial growth followed by Topsin-M (0.6 cm), Dithane M-45 (2.3 cm) and *Polygonium amplexicaule* (3.3 cm) at the same concentration. The present study illustrated the decrease in colony growth with the increase in concentration of fungicides. Sharma, 2006 found Mancozeb as the most effective fungicide against *Pestalotiopsis spp.* Taskeen-Un-Nisa *et al.*, (2011) reported maximum inhibition in mycelial growth was observed in the hexaconozole at 20% followed by other fungicides at the same concentration. Similar finding were reported by Patel *et al.* 2005 and Banyal *et al.*,2008.

Table 2: Effect of various concentrations of fungicides on radial growth of *Pestalotiopsis spp*.

Fungicides	Concentrations					
	Control	1%	5%	10%	15%	20%
F_1	6.9*a	6.7b	3.2hi	2.5l	2.9jk	2.3m
F_2	6.0c	3.3gh	2.8k	2.5l	1.2o	0.6q
F_3	6.7b	3.1ij	2.9j	1.7n	0.8p	0.0r
F_4	6.0c	5.0d	4.9d	4.2e	3.5f	3.3Fg
LSD $_{(0.05)}$ 0.15						
CV $_{(\%)}$	2.74%					

Response of *Rhizoctonia Spp* to various concentrations of fungicides and polygonium extract: Three fungicides and *Polygonium* extract namely, Dithane M-45, Ridomil Gold, and Topsin-M at five concentrations each was tested against *Rhizoctonia*. The results are accessible from Table 3. Among the four fungicides evaluated against *Rhizoctonia spp*, Ridomil Gold was found to be highly effective causing a significant reduction in mycelial growth of the fungus even in very low concentration of 1%. It completely inhibits the radial growth of Rhizoctonia at 15% and 20%. Other fungicides such as Topsin-M, Dithane M-45 and *Polygonium amplexicaule* have significant suppressive effect on growth of Rhizoctonia. Among all the fungicides Ridomil Gold has completely suppressed the radial growth of *Rhizoctonia* which is followed by Topsin-M (0.2 cm), Dithane M-45 (2.1 cm) and *Polygonium amplexicaule* (3.6 cm). There is considerable difference observed in the suppression of fungus growth with each step up increase in concentration from 1% to 20% of medium. The results were also supported by the studies of Sharma, 2006 & Sireesha and Venkateswarlu, 2013.

Table 3. Effect of various concentrations of fungicides on radial growth of *Rhizoctonia spp*.

Fungicides	Concentrations					
	Control	1%	5%	10%	15%	20%
F_1	9*a	4d	3.1g	4.6c	2.6h	2.1j
F_2	9a	3.1fg	2.4i	1.5l	0.5m	0.2
F_3	9a	2.5hi	1.9k	1.4l	0o	0o
F_4	9a	5.1b	4.6c	4.5c	3.3f	3.6e
LSD $_{(0.05)}$	0.21					
CV $_{(\%)}$	3.60%					

Table 4. Effect of various concentrations of fungicides on radial growth of *Fusarium spp*.

Fungicides	Concentrations					
	Control	1%	5%	10%	15%	20%
F_1	8.3*b	5.1c	5.2c	4.8d	3.3i	2.4k
F_2	8.8a	0m	0m	0m	0m	0m
F_3	8.7a	3.3i	2.9j	1.5l	0m	0m
F_4	8.8a	4.8d	4.6e	4.4f	3.9g	3.7h
LSD $_{(0.05)}$	0.08					
CV $_{(\%)}$	1.52%					

Table 5. Effect of various concentrations of fungicides on radial growth of *Fusarium spp.*

Fungicides	Concentrations					
	Control	1%	5%	10%	15%	20%
F_1	9*a	8.9a	7.8c	8.1b	3.6h	2j
F_2	9a	0l	0l	0l	0l	0l
F_3	9a	3.7h	3.4i	1.9k	0l	0l
F_4	9a	5.2d	5e	4.7f	3.7g	3.7g
$LSD_{(0.05)}$	0.06					
$CV_{(\%)}$	0.89%					

*Each value is the average of three replications

Where: F_1 = Dithane M-45 (Metalaxyl + Mancozeb), F_2 = Topsin-M (Mancozeb), F_3= Ridomil Gold (Thiophanate-methyl), F_4=*Polygonium amplexicaule* a botanical extract, LSD= Least significant difference, CV= Coefficient of Variance

They described that maximum inhibition in mycelial growth was observed in the Metalaxyl and Mancozeb at minimum concentration level followed by other fungicides.

Response of *Fusarium Spp* to various concentrations of fungicides and polygonium extract: Six fungicides at five concentrations effectively reduced the mycelial growth of *Fusarium spp* compared with control in which fungicides were not incorporated in the PDA medium. All doses of Topsin-M and 15% and 20% inhibited 100% mycelial growth of *Fusarium spp.* It is evident from the results that different fungicides used *in vitro* were effective on growth of *Fusarium spp*. There was a significant reduction in mycelial growth of all the concerned fungus during the study period with the increase in concentration of each fungicide. Four different fungicides at their different concentrations inhibited the mycelial growth of *Fusarium* with highest sensitivity recorded for Topsin-M and Ridomil Gold, while Dithane M-45 and Polygonium amplexicaule (botanical extract) exhibited an intermediate effectiveness in controlling mycelial growth as shown in Table 5. Bajwa and Javaid 2007, Khanzada *et al.* 2005 also found that these four fungicides were the most effective fungicides that inhibited the radial growth of Fusarium.

References

Bajwa, R. and A. Javaid. 2007. Integrated disease management to control Shisham (*Dalbergia sissoo* Roxb.) decline in Pakistan. Pak. J. Bot. 39: 2651-2656.

Banyal, D.K., V. Mankotia and S.K. Sugha. 2008. Integrated management of tomato collar rot caused by *Sclerotium rolfsii*. J. Mycol. Pl. Path. 38: 165–167.

Bloomberg, W.J. 1981. "Disease caused by *Fusarium* in Forest Nurseries". Pages 178-187. *In*: Fusarium: Diseases, Biology, and Taxonomy (P. E.Nelson, T. A. Toussoun, and R. J. Cook, Eds.). Pennsylvania State: University, University Park.

Brown, A., S. Green and S. Hendry. 2005. Needle diseases of pine. Forestry Commission Information Note 67. Forestry Commission, Edinburgh.

Carlucci. A., L. Colatruglio and S. Frisullo. 2007. First report of pitch canker caused by *Fusarium circinatum* of *Pinus halepensis* and *P. pinea* in Apulia. Pl. Dis. 91:1683

Gupta B. and B. Dass . 2007. Composition of herbage in *Pinus roxburghii* Sargent stands: basal area and Importance value index. Caspian J. Environmental Sciences 5: 93–98.

Huang, J.W. and E.G. Kuhlman. 1990. Fungi Associated with Damping off of Slash Pine Seedlings in Georgia. Plant Dis. 74:27-30.

Jones, R.K. and C.W. Averre. 2000. Damping-off in Flower and Vegetable Seedlings. Plant Pathology Extension: North Carolina State University Ornamental Disease Note. No. 14.

Khanzada, M.A., A.M. Lodhi and S. Shahzad. 2005. Chemical control of *Lasiodiplodia theobromae*, the causal agent of mango decline in Sindh. Pak. J. Bot. 37: 1023-1030.

Lori, G., M.I. Salerno, S. Wolcan, J. Gimenez and G. Basil. 1999. Fusarium Species from a Forest Nursery Soil in Western Patagonia And Reduction of their Population by Soil Solarization. J. Plant Dis. and Prot. 106: 363-371.

Patel, N.A., S.R.S. Dange and S.I. Patel. 2005. Efficacy of chemicals in controlling fruit rot of tomato caused by *Alternaria solani*. Indian J. Agri. Res., 39:507.

Sharma, R.L. 2006. Efficacy of fungicide impregnated paper liners against storage rot of tomato fruit. J. Mycol. Plant Pathol. 26: 310-311.

Siddiqui, M.F., M. Ahmed, M. Wahab and N. Khan. 2009. Phytosociology and structure of *Pinus roxburghii* Sargent (Chir Pine) in lesser Himalayan and Hindukush range of Pakistan. Pak. J. Bot. 41: 2357–2369.

Singh, S.N., B.P. Yadav, S.K. Sinha and K.L. Ojha. 1997. Efficacy of plant extract in inhibition of radial growth of *Colletotrichum capsici*. J. Appl. Biol. 51: 180-183.

Sireesha, O. and N. Venkateswarlu. 2013. In vitro evaluation of botanicals and panchagavya against leaf blast fungus *Pyricularia grisea*. Asian J. Pharmacy Clin. Res. 6: 84-86

Sturrock, R.N., S.J. Frankel, A.V. Brown, P.E. Hennon, H.T. Kliejunas. K.J. Lewise, J.J. Worrall and A.J. Woods. 2011. Climate Change. For. Dis. 60: 133-149.

Taskeen-Un-Nisa, A.H. Wani, M.Y. Bhat, S.A. Pala and R.A. Mir. 2011. In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*. J. Biopest. 4: 53–56.