

Survey, pathogenicity and molecular characterization of root pathogens associated with root rot of *Cedrus deodara*(Roxb.) G. Don in district Solan, Himachal Pradesh, India

ABSTRACT

Root rot disease is one of the most serious diseases that significantly reduces the plant's productivity, commonly known as collar rot, foot rot, or damping off. The main symptom of this condition is damping off. The newly emerging seedlings collapse and die in areas with severe infection, and the majority of seedlings may die before or after emergence. Due to Root rot disease in *C. Deodara* which is our state tree, the damage caused by it to the seedlings there was urgent need to identify and study the disease so as to develop proper management strategies. In Himachal Pradesh, a field survey was carried out to know the disease incidence of root rot pathogen in the *Cedrus* growing regions in district Solan. The survey included the Kanaghat, Solan, and Chail mountains. The Kandaghat range covered Kiari, Karol, and Bisha nurseries, the Solan range Shilli, Nauri nurseries, and the Chail range Chiunth, Chail, and Gaura nurseries were all covered. During the survey of root rot pathogen of *Cedrus deodara*, 10.90 to 62.15% incidence was recorded in district Solan nurseries. Maximum incidence of the root rot was recorded in Kandaghat range (37.35%) followed by Solan range (33.85%) and Chail range (30.93%), respectively. Amongst nurseries found that, Bisha nursery had maximum incidence (62.15%) followed by Nauri (48.46%) and Chiunth nurseries (44.65), respectively. Cultural and morphological characteristics of root rot pathogen were studied and identified as *Rhizoctonia solani*. ITS1/ITS4 markers were employed for the molecular identification of the pathogen that caused the root rot in *Cedrus deodara*, which was confirmed to be binucleate *Rhizoctonia* AG-E. For the root rot pathogen, isolation frequency was also observed in three solan ranges. Isolation frequency of Chail range comprising of two ranges showed the maximum of 37.71% followed by Kandaghat (34.42%) and Solan (19.37%), respectively. Numerous pathogens were isolated, however, three pathogen, binucleate *Rhizoctonia* AG-E, *Fusarium oxysporum*, and *Phoma exigua* were found to occur most frequently. Binucleate *Rhizoctonia* AG-E was the most often isolated of the three primary pathogens, occurring at a maximum frequency of 50.23%, followed by *P. exigua* (18.98%) and *F. oxysporum* (7.49%). Three different methods were used to test the pathogenicity of binucleate *Rhizoctonia* AG -E for their ability to produce root rot symptoms.

The soil infestation inoculation method recorded the highest disease incidence (83.48%) followed by the root dip (34.53%) and stem application (23.76%), respectively.

Keywords: binucleate *Rhizoctonia*, isolation frequency, pathogenicity, root rot, survey.

INTRODUCTION

In the western Himalayas and on the slopes of the Hindu Kush, *Cedrus deodara* (Roxb.) G. Don, known as Himalayan *Cedrus*, grows at heights between 1100 and 3000 metres. Young *Cedrus* seedlings are usually prone to pathogen infections during the early phases of plant establishment, particularly those that result in root rot and wilt diseases. Root rot fungi that are particularly dangerous to forest nurseries include *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, and *Macrophomina* (Tomar and Thakur, 2020). According to Dar *et al.*, several diseases of *Abies pindrow* and *Pinus wallichiana* seedlings are caused by *Fusarium* sp. and *R. solani* in Kashmir (2011). Chakravarty and Mishra also found *Rhizoctonia solani* as a root rot pathogen in *Quercus* seedlings (2007). In many parts of the world, including Iraq, the fungus *Rhizoctonia solani* is regarded as the most significant in affecting the crop, resulting in significant economic losses on the crop. *Rhizoctonia* is a widespread, destructive and versatile plant pathogen, are distributed worldwide in both agriculture and forest soils and are known to cause root diseases of several crop plants (Yadav *et al.*, 2022).

One of the most serious diseases that significantly reduces the plant's productivity is *Rhizoctonia solani*-caused root rot, commonly known as collar rot, foot rot, or damping off. The main symptom of this condition is damping off. The newly emerging seedlings collapse and die in areas with severe infection, and the majority of seedlings may die before or after emergence. They may also develop reddish-brown cankers and foot rot on the root and stem that are near to the ground. Although, it can also infect other plant sections such the green foliage, seeds, and hypocotyls (Acharya *et al.*, 2014). Infected plants have poorly developed roots; smaller roots are either missing altogether or have decayed. Plants that exhibit stunted development are simple to remove. Root rot causes significant losses (Yadav *et al.*, 2020).

The fungus attacks the plant during its growth stages, damping off seedlings, rotting seeds before and after the bud stage, as well as the rotting of the roots and the bases of the stems. The fungus is distinguished by the development of a brown-colored fungus, thick walls that branch at semi-right angles with some shortenings present, and transverse barriers (Mohammed *et al.*, 2022). The host is killed by these infections, which result in post-emergence damping-off or root rot/wilt in terminal unbarized roots and young seedlings. Root rot fungi, which represent a serious threat to nurseries, restrict the generation of dry matter and specifically injure plants, according to Aigbe and Remison (2010). Favorable circumstances include abundant rainfall, high spring and summer temperatures, and weak soil structure promote the growth of pathogen fungal colonies.

MATERIAL AND METHODS

Disease survey

During the months of June, July, and August, surveys were done in various forest nurseries around the district of Solan, Himachal Pradesh in year 2017 & 2018. Kandaghat range (Kiari, Karol, Bisha nurseries), Chail range (Chiunth and Gaura nurseries), and Solan range (Kiari, Karol, Bisha nurseries) were among the nurseries assessed (Shilli and Nauni nurseries). The diseased nursery plants were photographed and evaluated for symptom progression after being collected in paper bags. Calculation of disease incidence by using a formula.

$$\text{Disease} \quad : \quad \frac{\text{Number of diseased plants}}{\quad} \times 100$$

incidence % $\frac{\text{Total number of plants examined}}{\text{Total number of plants examined}}$

The infected samples were kept in paper bags in the refrigerator at 5°C for isolation, identification, and further research.

Isolation and maintenance of the pathogen

Potato-Dextrose-Agar medium having following composition was prepared by using method described by Johnston and Booth (1983). Composition of Potato-Dextrose-Agar medium- potato 200g, destrose 20g, agar agar 20g and distilled water 1lt.

To remove the surface soil, the diseased plant material was rinsed with running tap water. With the use of a sterilised blade and laminar air flow, little parts of 1 to 2 mm size were extracted from the infected and healthy portions of the plant's roots and collar region. Under aseptic conditions, these bits were surface sterilised for 30 seconds with Sodium hypochlorite (0.05 percent) and washed three times with sterilised distilled water. After removing excess moisture using sterilised filter paper, the bits were put to sterilised Petri plates containing potato dextrose agar (PDA) medium. These Petri plates were later incubated at 27±1°C in BOD incubator and examined daily for mycelial growth. The fungal growth developed in Petri plates was purified by hyphal tip technique. The culture slants were preserved at 5°C in refrigerator and subcultured fortnightly.

Pathogenicity test

Under pot culture conditions, the causative organism's pathogenicity was tested using standard Koch's postulates. The sterilised soil was placed in 12 inch diameter earthen pots. A week before planting one month old rooted deodar seedlings, 50 g of mass cultured pathogen media was added to these pots. In total, three Deodar seedlings were grown in each container at the proper distance. In this experiment, thirty pots were utilised, and the control was maintained without the addition of the causal fungus. The fungus was re-isolated from the seedlings that had been infected. A phase contrast stereoscopic microscope was used to determine the same's identify.

Multiplication in Potato dextrose broth (PDB)

In 250ml conical flasks, five mm discs of a seven-day-old binucleate *Rhizoctonia* culture were introduced to 100ml PDB. These flasks were shaken for 14 days at 80-100 rpm at $26 \pm 1^\circ\text{C}$ temperature on a rotary shaker.

The pathogen's mycelial mat produced on PDB was separated using a sieve and rinsed with sterilized distilled water many times. By gently pressing on the mycelium, excess water was evacuated. One litre of sterilised distilled water was added to five grammes of this mycelial mat, which was constantly swirled. Haemocytometer was used to determine the number of colony forming units. The homogenised suspension was then utilised for inoculation right away.

Preparation of mass culture of the root rot pathogen

On a sand-wheat medium, the root rot pathogen was mass grown (1:2). The wheat grains were just cooked long enough to soften them. The excess water was drained and the wheat grains were dried by air. The wheat grains were then combined with sand (two parts wheat grain to one part sand) and 2% sucrose. The medium was then loaded into autoclavable polypropylene bags (150 g per bag), plugged with non-absorbent cotton, and autoclaved twice at 15 pounds per square inch pressure for 1 hour each time.

Under aseptic circumstances, the sterilized medium was inoculated with a one-week-old culture of the isolated pathogen. In each bag, five to six pieces of this fungus with a diameter of 4 mm were inserted. The bags were shaken on a regular basis to ensure that the culture expanded evenly. The inoculation bags were incubated for 20 days at $27 \pm 1^\circ\text{C}$ in a BOD incubator, and the mass culture was utilized for several studies.

Soil infestation

Pathogen mass multiplied on Wheat grain was uniformly put to the polybags filled with sterilized soil. The pots were immediately sprayed with water after being inoculated. For incubation, the bags were placed in a glasshouse for seven days. Seven days after soil inoculation, one-month-old *Cedrus* seedlings were planted. Ten seedlings were replicated three times in the treatment. The seedlings were checked for symptom manifestation on a daily basis. The disease's usual symptoms were documented. Total number of seedlings and number of infected seedlings were recorded to calculate per cent disease incidence (PDI).

PDI was calculated by adopting following formula:

$$\text{PDI} = \frac{\text{Number of plants affected}}{\text{Total number of plants}} \times 100$$

Total number of plants

The pathogen was re-isolated from infected seedlings, and the resulting culture was compared to the original culture.

Root dip inoculation

Cedrus deodara seedlings were grown in sterilised soil in plastic bags (15cm diameter). One-month-old seedlings were carefully plucked from their pots, cleaned with sterilised distilled water to eliminate any excess soil on the root surface, and the root system's distal one-third was trimmed. These clipped seedlings were dipped in the pathogen's mycelial suspension for 10 minutes before being transplanted in the same poly bags. The mycelial suspension was poured over the seedlings' root zones. Ten seedlings were replicated three times in the treatment. Seedlings were watered on a regular basis and monitored for symptom development.

The pathogen was reisolated from the infected seedlings and compared to the original culture. The total number of seedlings and the number of seedlings that were affected were recorded in order to calculate the percent disease incidence, which was done using the formula indicated earlier.

Stem application

One month old Seedlings of *Cedrus deodara* were grown in poly bags (15cm diameter) filled with sterilized soil. The collar portion of the seedling was pricked three times with a sterile needle. The mycelial suspension was applied with a camel hair brush and remaining mycelial suspension was placed to the soil @ 5ml per pot. The inoculated stem parts were wrapped in blotter papers immediately after inoculation, and the pots were watered regularly. The blotter paper wrappers were removed two days after inoculation, and the seedlings were inspected daily for symptoms

Ten seedlings were replicated three times in the treatment. The pathogen was re-isolated from infected seedlings, and the resulting culture was compared to the original. To compute the percent disease incidence, the total number of seedlings and the number of afflicted seedlings were recorded. The control bags were kept as is, with no pathogen inoculum added, and were watered on a regular basis.

RESULTS AND DISCUSSION

Survey of root rot of *Cedrus deodara* in different parts of district Solan:

Disease Incidence of *Cedrus deodara* root rot was recorded during the cropping season of 2017-2018 and 2018-2019 in different *Cedrus deodara* growing nurseries of Solan range (Table 1) (Plate 1) (Fig.1). Root rot Incidence recorded maximum in Kandaghat range (37.35%) followed by Solan (33.85) and Chail range (30.93%), respectively. Amongst nurseries, Bisha nursery had maximum incidence (62.15%) followed by Nauni (48.46%) and Chiunth nurseries (44.65), respectively. The overall mean of different years suggested that disease was 5.56 per cent more in the year 2018 -19 than the previous year. Bisha, Nauni and Chiunth nurseries were worst affected with root rot disease. Overall, disease incidence ranged between 10.90 to 62.15 per cent in different locations of *Cedrus deodara* nurseries. Dar and coworkers (2017) while working on root rot of *Abies pindrow* in Kashmir valley recorded 29.3 and 32.2 per cent disease incidence for two consecutive years. Similarly, Dar and his associates while working on root rot of *Pinus wallichiana* during 2011 recorded 4 – 5 to 6.75 per cent disease incidence in various nurseries of Kashmir valley. **Plate 1, Table 1**

Plate 1. Disease incidence of root rot in seedlings of *Cedrus deodara* in different nurseries



Seedling infected with root rot disease

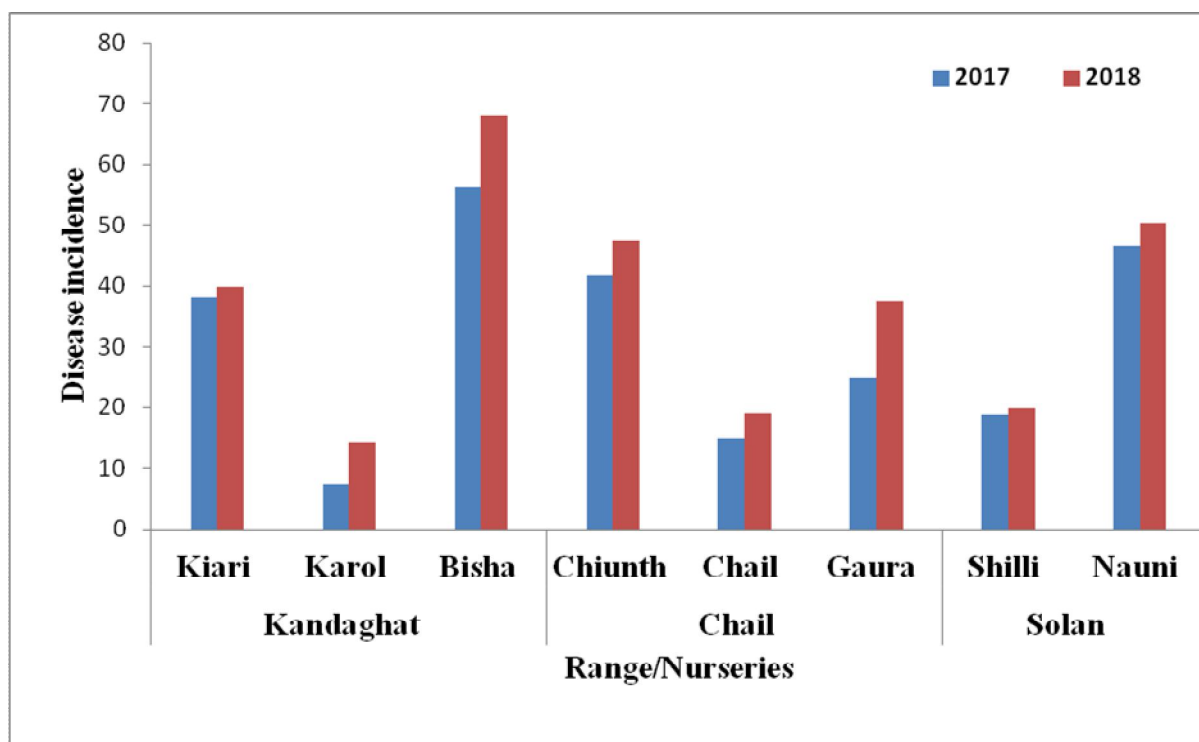


Healthy nursery

Table 1. Disease incidence of root rot in seedlings of *Cedrus deodarain* district Solan

Range	Root rot incidence (%) in the year			Pooled mean
	Nursery	2017	2018	
Kandaghat	Kiari	38.10	39.90	39.00
	Karol	7.50	14.30	10.90
	Bisha	56.30	68.00	62.15
Mean		33.96	40.73	37.35
Chail	Chiunth	41.80	47.50	44.65
	Chail	15.00	18.90	16.95
	Gaura	24.90	37.50	31.20
Mean		27.23	34.63	30.93
Solan	Shilli	18.70	19.8	19.25
	Nauni	46.50	50.43	48.46
Mean		32.60	35.11	33.85
Overall Mean		31.26	36.82	

Fig.1. Graphical representation of disease incidence of root rot in seedlings of *Cedrus deodara*

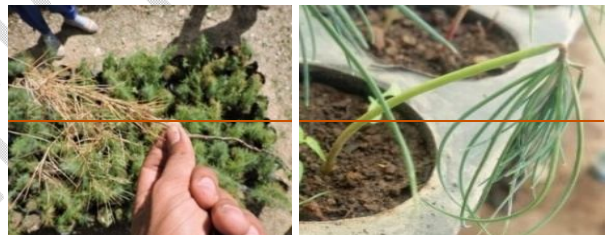


Isolation frequency

After the samples collection from diseased nurseries of Solan district in Himachal Pradesh. Mean isolation frequency was determined as number of isolates of selected fungus from 15 sampled root and collar portion of diseased seedlings. The results in Table 1 indicated that out of the three ranges surveyed, many pathogens were isolated viz., *Fusarium oxysporum*, *Rhizoctonia* spp, *Phoma* spp, *Phytophthora* spp, *Mucor* spp, and *Trichoderma* spp. However, the maximum frequency was found to be of *Rhizoctonia* spp., *Fusarium oxysporum* and *Phoma exigua* (Plate 1). The identity of three pathogens was confirmed from National Centre of Fungal taxonomy, New Delhi. *Rhizoctonia* spp. was isolated from all the nurseries whereas *Fusarium oxysporum* and *Phoma exigua* were absent in some of them. The results presented in Table 1 indicated that out of the three ranges Chail range comprising of two nurseries showed the maximum isolation frequency of 37.71 per cent followed by Kandaghat (34.42%) and Solan (19.37%), respectively. Amongst the three major pathogens isolated *Rhizoctonia* spp. was most frequently isolated with a maximum frequency of 50.23 per cent followed by *Phoma exigua*(18.98%) and *Fusarium oxysporum*(7.49%), respectively (Fig 2). Isolation frequency of *Phoma exigua* and *Fusarium oxysporum* were significantly lesser as compared to *Rhizoctonia*

spp. The results also revealed that population of *Rhizoctonia* was equally higher in root as well as collar portion whereas other two pathogens were isolated more from the root portions. In his studies on isolation of fungus from roots of *Abies pindrow*, Dar *et al.* (2017) also revealed the presence of various pathogens viz., *Fusarium oxysporum* f.sp. *pini*, *Rhizoctonia solani*, *Sclerotium rolfsii* and *Pythium* sp. Ahanger *et al.* (2011) were of the opinion that *F. oxysporum* was 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 which is contrary to the results presented here as isolation frequency of *Rhizoctonia* sp. was more as compared to *Fusarium* sp. However, Work done by Lilja *et al.* (2010) is in consonance with the present study where population of *Rhizoctonia solani* in many forest nurseries was more than other soil borne pathogen. **Plate 2, Table 2**

Plate 2. Symptoms of root rot of *Cedrus deodaran* seedlings



- i) Post emergence damping off of seedlings ii) Drying and drooping down seedling symptom of seedlings**



- iii) Complete rotting of stem and drooping of seedling**

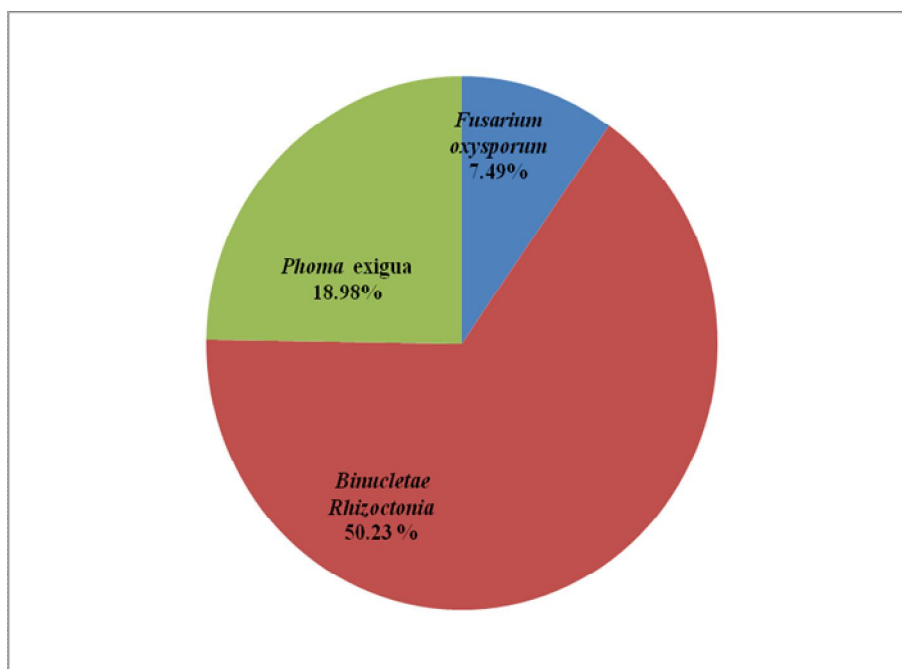
Table 2. Isolation frequency (%) of main pathogens associated with root rot of *Cedrus deodara*

Range/ Nursery		Isolation frequency (%)												Mean	Pooled mean
		<i>Fusarium</i> spp.				<i>Rhizoctonia</i> spp.				<i>Phoma</i> spp.					
		2017		2018		2017		2018		2017		2018			
		Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root		
Solani	Nauni	6.67 (14.23)	12.50 (20.43)	5.83 (13.47)	10.83 (19.03)	45.83 (42.57)	75.83 (61.65)	8.33 (16.73)	5.00 (10.59)	4.17 (11.35)	24.17 (29.35)	1.67 (4.30)	30 (33.14)	19.23 (23.07)	19.37 (22.62)
	Shilli	5.83 (13.90)	8.33 (15.92)	4.17 (11.64)	9.17 (16.76)	70.00 (57.14)	80.00 (63.63)	2.50 (7.33)	4.16 (9.60)	0.83 (3.03)	14.17 (21.97)	4.17 (11.64)	30.83 (33.60)	19.51 (22.18)	
Kandaghat	Kiari	6.67 (14.89)	6.66 (14.75)	4.17 (9.60)	5.83 (13.62)	42.50 (40.60)	42.50 (40.60)	58.33 (49.78)	74.17 (59.50)	4.17 (9.60)	21.67 (27.43)	1.67 (4.30)	25.83 (29.79)	24.51 (26.20)	34.42 (32.81)
	Karol	4.17 (9.60)	3.33 (10.36)	2.50 (7.33)	6.67 (14.89)	25.83 (30.45)	25.83 (30.45)	15.83 (23.42)	21.67 (27.63)	2.50 (5.29)	4.17 (11.35)	1.67 (4.30)	6.67 (14.89)	10.06 (15.83)	
	Bisha	10.00 (18.42)	8.33 (15.92)	4.17 (9.60)	9.17 (17.19)	75.00 (60.09)	75.00 (60.09)	77.50 (62.53)	83.33 (66.17)	1.67 (4.30)	6.67 (14.47)	3.33 (8.61)	9.17 (17.34)	30.27 (29.58)	
Chail	Chiunth	6.67 (14.75)	11.66 (19.78)	5.83 (13.47)	6.67 (14.89)	49.17 (44.50)	49.17 (44.50)	61.67 (52.11)	75.83 (61.17)	4.17 (9.17)	7.50 (15.74)	1.67 (4.30)	25.83 (29.79)	25.48 (27.08)	37.71 (35.45)
	Gaura	5.00 (12.63)	19.16 (25.94)	1.67 (4.30)	18.33 (25.26)	55.00 (47.85)	55.00 (47.85)	68.33 (56.08)	83.33 (66.53)	55.00 (47.85)	86.67 (68.91)	68.33 (56.08)	83.33 (66.53)	49.93 (43.82)	
Mean		6.42	10.00	4.04	9.52	51.90	57.61	41.78	49.64	10.35	23.57	11.78	30.23		

		(14.06)	(17.58)	(9.92)	(17.38)	(46.17)	(49.82)	(38.28)	(43.06)	(12.94)	(27.03)	(13.36)	(32.15)		
Overall Mean		7.49 (14.73)				50.23 (44.33)				18.98 (21.36)					
C.D_{0.05}	Nurseries = 2.80, Pathogen = 4.66, Nurseries x Pathogen = 9.70														

UNDER PEER REVIEW

Fig.2 Graphical representation of isolation frequency (%) of main pathogens associated with root rot of *Cedrus deodara*



Isolation frequency (%)

Fungal isolation

Culture of fungus Isolate from diseased roots and collar region of root rot seedlings on the potato dextrose agar (PDA) medium using standard isolation techniques. Purification of isolated fungus by hyphal tip method on PDA Petri plates and incubated at 25°C. Fungal culture regularly monitored and maintained by sub culturing after every 15 to 25 days.

Identification of the pathogen

Fungus attain growth in 3-4days with fluffy white mycelium. Mycelium septate, right angle branched and size of hyphae between 6.2-6.4µm. Mycelium turn pale to brown with maturity. Sclerotia formed within 55 days in culture with dark brown, round, rough and measured 0.75-3.0 mm in diameter. Brown and barrel shaped Monilioid cells arising from the sclerotia with measured 15-38 × 10-15µmin size.

Molecular confirmation of the pathogen

After morphological identification for conforming identification of pathogen molecular sequencing was outsourced from Eurofins Genomics, Bengaluru by using ITS1/ITS4 markers

(Khan *et al.*, 2021; Khan and Javaid, 2022), yielded 706 bp amplicons (Plate 3) (Fig.3) identified as binucleate *Rhizoctonia* AG –E with a telemorphic stage *Ceratobasidium* sp. group CAG 6 and sequence was submitted to gene bank (Tomar and Thakur 2020; Thakur and Tomar 2020).

Fig.3 Phylogenetic tree based on ITS sequences drawn using the maximum-likelihood method showing relationship between the isolate of binucleate *Rhizoctonia* AG-E (MK226512.1) and similar other sequences deposited in the NCBI Gen Bank

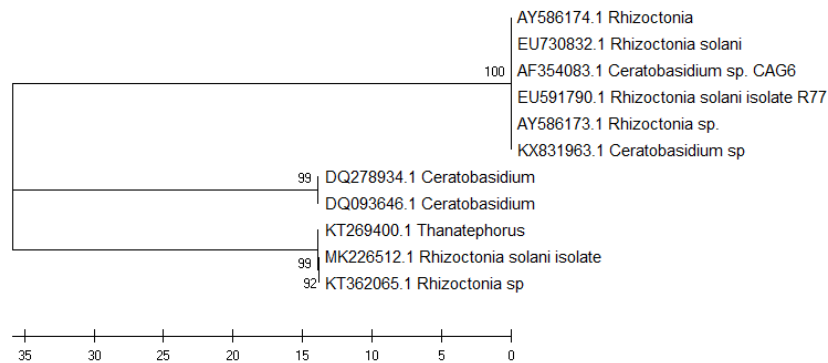
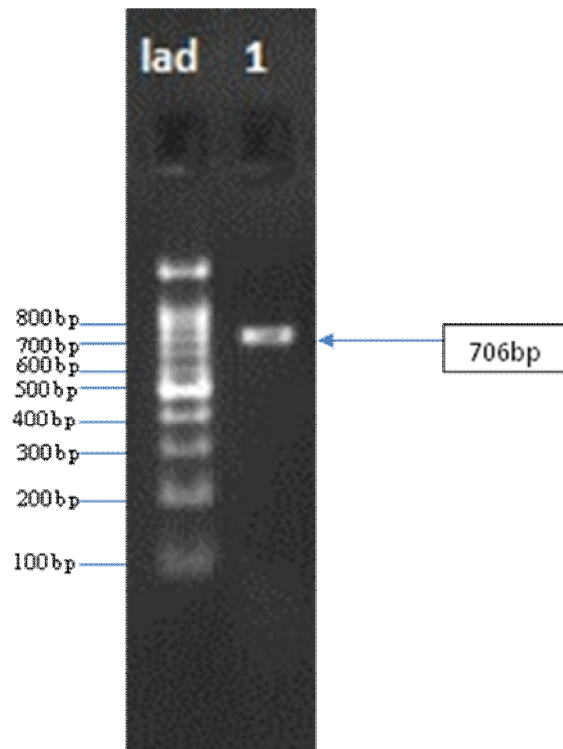


Plate 3. Agarose gel photograph of 706 bp amplicon of binucleate *Rhizoctonia* AG-E (Courtesy: Eurofins Genomics)

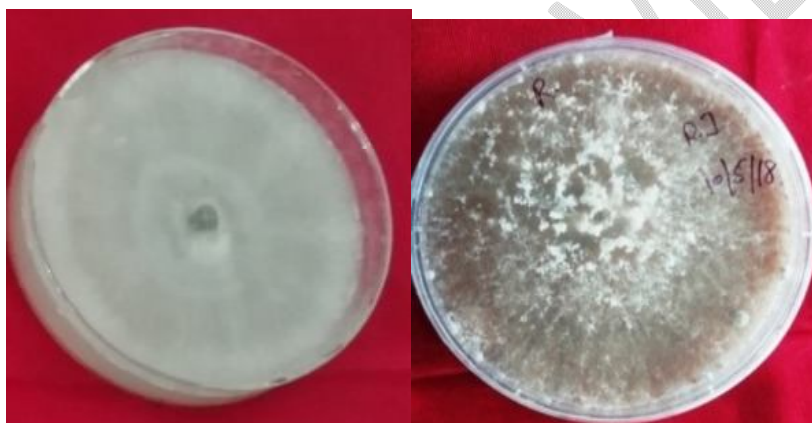


Pathogenicity test

The pathogenicity test of the isolated fungus was conducted on one month old rooted cuttings of *Cedrus deodara* by standard Koch's postulates in nursery bags. Most frequently isolated three main fungi viz., binucleate *Rhizoctonia* AG-E, *F. oxysporum* and *P. exigua* were put to pathogenicity test to ascertain the causal organism of root rot in seedlings of *C. deodara*. The results present in Table 2 revealed that maximum incidence (47.53%) was recorded in seedlings inoculated with binucleate *Rhizoctonia* AG-E followed by *Fusarium oxysporum* (3.55%) and *Phoma exigua* (0.00%), respectively (Fig 4). Moreover, the root rot symptoms were conspicuous in the seedlings inoculated with binucleate *Rhizoctonia* AG-E within 25 days of inoculation whereas root rot symptoms were negligible in case of *Fusarium oxysporum* and totally absent in case of *P. exigua*. *F. oxysporum* produced wilting and needle chlorosis along with slight vascular discoloration after 35 days of inoculation (Plate 2) while *P. exigua* exhibited no root rot symptoms at all. Root rot symptoms like thinning and blackening of roots, rotting at collar porting, damping off were recorded on seedlings inoculated with binucleate *Rhizoctonia* AG-E. All the pathogens were reisolated from the inoculated seedlings and hence due to root rot symptom production like extensive root decay, absence or lack of lateral roots, cortical tissue

decay, stunted growth and needle discolouration, binucleate *Rhizoctonia* AG-E, was used for further studies as the main pathogen of root rot of *Cedrus deodara*. While working on stem blight of *Catharanthus rosea* (*V. rosea*, *Vinca minor*) and *Vinca major* (big-leaf or variegated) caused by the fungus *Phoma exigua*, Scott and Broadhurst (2011) reported rapidly expanding, dark brown to black, girdling lesions on stems at the soil line which resulted in dieback. Similar symptoms of root rot by *Rhizoctonia solani* were reported by various workers while working on root rot of *Pinus wallichiana*, *Abies pindrow* and Urd bean (Rana and Tripathi, 1983; Ahangar *et al.*, 2011; Dar *et al.*, 2013). **Plate 4, Table 3**

Plate 4: Plates of Rhizoctonia culture.



a) Pure culture of *Rhizoctonia*

b) Sclerotia formation in culture

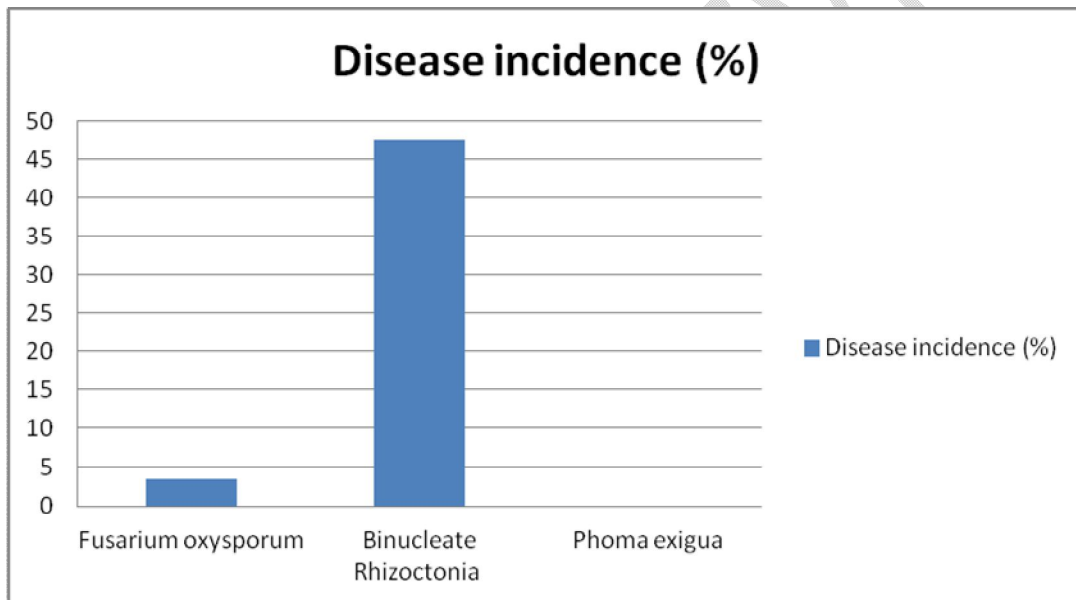


b) Monilioid cells of *Rhizoctonia* under compound microscope

Table 3. Per cent disease incidence of root rot of *Cedrus deodara* by three frequently isolated fungi under *in vitro* conditions

Fungi	Disease Incidence (%)
<i>Fusarium oxysporum</i>	3.55
<i>Rhizoctonia</i> spp.	47.53
<i>Phomaexigua</i>	0.00
CD0.05	1.21

Fig 4 Graphical representation of Per cent Disease Incidence of root rot of *Cedrus deodara* by three frequently isolated fungi under *in vitro* conditions



Performance of three different inoculation methods in proving pathogenicity of binucleate *Rhizoctonia* AG –E

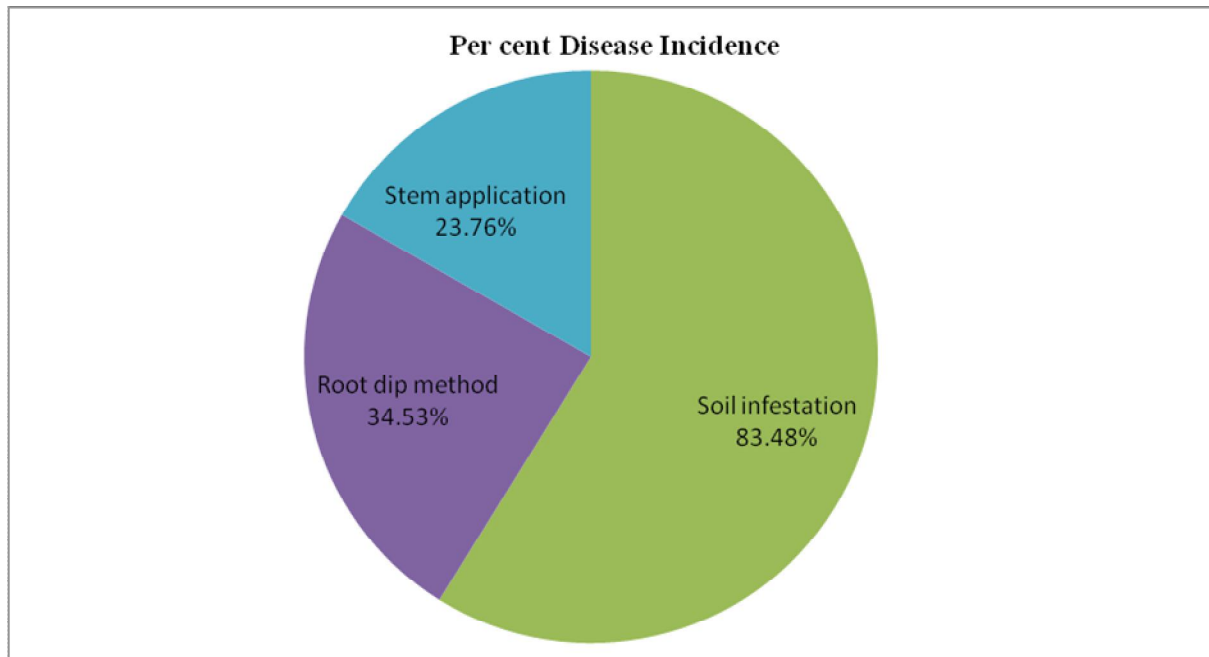
Pathogenicity of binucleate *Rhizoctonia* AG –E was proven by three different methods to test their efficacy in producing root rot symptoms. The data presented in Table 4 clearly depicted that all the inoculation methods were found to be effective in producing root rot symptoms. However, the diseases incidence was maximum (83.48%) in soil infestation inoculation method followed by root dip (34.53%) and stem application method (23.76%) respectively (Fig 5). Soil infestation method resulted in pre- damping off symptoms. The typical symptoms were observed as rotting of emerging seedlings in soil and development of necrotic lesions at collar region of

the seedlings. These symptoms lead to death of seedlings due to collapse of such seedlings. The pathogen was reisolated from the infected seedlings and the culture obtained was found to be similar with the original culture in all respects. The soil infestation method resulted in higher disease incidence due to proper establishment of culture in soil which lead to the vigorous symptom production and death of seedlings within 15 days of inoculation. In case of root dip and stem application method symptoms appeared after 28 and 33 days of inoculation, respectively and delayed the death and collapse of seedlings. The control plants showed no sign of root rot at all as no pathogen was inoculated in the soil. The pathogen was reisolated from the infected seedlings and the culture obtained was found to be similar with the original culture in all respects. The results are in agreement with studies of Jiskani *et al.* (2007) who confirmed *R. solani* as the predominant damping off causing fungus while conducting an experiment to prove its pathogenicity on tomato through soil infestation method. These findings are also supported by the observations made by Gordon *et al.*, 2005 who reported the superiority of the soil infestation method over root dip inoculation method while working with a soil borne fungal pathogens.

Table 4. Performance of inoculation methods in proving pathogenicity of *Rhizoctonia* spp.

Method of inoculation	Per cent Disease Incidence (%)
Soil infestation	83.48
Root dip method	34.53
Stem application	23.76
Control	0.00
CD 0.05	5.68

Fig 5. Graphical representation of Performance of inoculation methods in proving pathogenicity of binucleate *Rhizoctonia* AG-E



SUMMARY AND CONCLUSION

In Himachal Pradesh survey was conducted in different *Cedrus* growing areas of district Solan. Disease Incidence varies between 10.90 to 62.15 per cent in different locations. Incidence found in all surveyed locations but maximum incidence of the root rot was recorded Kandaghat (37.35%) followed by Solan range (33.85%) and Chail range (30.93%). Amongst nurseries surveyed, Bisha nursery had maximum incidence (62.15%) followed by Nauri (48.46%) and Chiunth nurseries (44.65). The symptoms of root rot in *Cedrus* seedlings varied from seed rot, hypocotyl rot and collar rot to root rot. On the basis of morphological characters isolated fungus identified as *Rhizoctonia solani*. The cultures were sent to National Centre of fungal taxonomy, New Delhi for the confirmation of the identity. To ensure the identity of the fungus, molecular sequencing was outsourced from Eurofin Genomics, Bengaluru. The ITS region was amplified by using ITS1 and ITS4 primers and pathogen identified as binucleate *Rhizoctonia* AG –E = *Ceratobasidium* sp. CAG 6. Isolation frequency of three ranges was recorded out of three ranges Chail range comprising of two nurseries showed the maximum isolation frequency of 37.71 per cent followed by Kandaghat (34.42%) and Solan (19.37%), respectively. Many pathogens were isolated viz., *Fusarium oxysporum*, *Rhizoctonia* spp, *Phoma* spp, *Phytophthora* spp, *Mucor* spp, and *Trichoderma* spp. However, the maximum frequency was found to be of *Rhizoctonia* spp., *Fusarium oxysporum* and *Phoma exigua*. Amongst the three major pathogens isolated *Rhizoctonia* spp. was most frequently isolated with a maximum frequency of 50.23 per cent

binucleate *Rhizoctonia* AG –E followed by *Phoma exigua*(18.98%) and *Fusarium oxysporum*(7.49%), respectively. Pathogenicity of binucleate *Rhizoctonia* AG –E was proven by three different methods to test their efficacy in producing root rot symptoms, maximum disease incidence (83.48%) recorded in soil infestation inoculation method followed by root dip (34.53%) and stem application method (23.76%) respectively.

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