Survey, isolation frequency and pathogenicityof root rot pathogens of *Cedrusdeodara*(Roxb.) G. Don in district Solan, Himachal Pradesh

ABSTRACT

During the survey of root rot pathogen of *Cedrusdeodara*, 10.90 to 62.15%~~per cent~~ incidence was recorded in district Solan ~~*Cedrus deodara*~~nurseries. In Kandaghat range Kiari, Karol and Bisha nurseries, Solan range Shilli, Nauni nurseries and Chail range Chiunth, Chail and Gaura nurseries covered. Maximum incidence of the root rot was recorded in Kandaghat (37.35%) followed by Solan range (33.85%) and Chail range (30.93%), respectively. Amongst nurseries surveyed, Bisha nursery had maximum incidence (62.15%) followed by Nauni (48.46%) and Chiunth nurseries (44.65), respectively. Cultural and morphological characteristic~~t~~s of root rot pathogen were studied ~~by which it~~and identified as *Rhizoctonia solani*. For the confirmation, molecular identification ~~also done~~ of the root rot pathogen ~~of *Cedrus deodara* caused by~~ binucleate *Rhizoctonia* AG-E was also done. Isolation frequency of 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, maximum frequency was found to be of binucleate *Rhizoctonia* AG-E, *Fusarium oxysporum*and*Phomaexigua*. Amongst the three major pathogens isolated, binucleate *Rhizoctonia* AG-E was the most frequently ~~isolated~~ occurring with a maximum frequency of 50.23%~~per cent~~ binucleate *Rhizoctonia* AG –E followed by ~~*Phoma*~~ *P. exigua*(18.98%) and ~~*Fusarium*~~ *F. 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.

Keywords: Root rot, survey, isolation frequency, pathogenicity, binucleate *Rhizoctonia*

INTRODUCTION

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In the western Himalayas and on the slopes of the Hindu Kush, *Cedrusdeodara* (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 *Abiespindrow* 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).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.

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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. 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.

$$\text{Disease incidence \%} : \frac{\text{Number of diseased plants}}{\text{Total number of plants examined}} \times 100$$

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

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Potato-Dextrose-Agar medium having following composition was prepared by using method described by Johnston and Booth (1983).

Potato	-	200.0 g
Dextrose	-	20.0 g
Agar-agar	-	20.0 g
Water	-	1000 ml

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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 ±1°C temperature on a rotary shaker.

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Preparation of mycelial suspension

The pathogen's mycelial mat produced on PDB was separated using a sieve and rinsed with sterlized 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.

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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±1°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 sterlized 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:

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

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

Root dip inoculation

Cedrusdeodara 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 *Cedrusdeodara*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 *Cedrusdeodara*in different parts of district Solan:

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Disease Incidence of *Cedrusdeodara*root rot wasrecorded during the cropping season of 2017-2018 and 2018-2019 in different *Cedrusdeodara*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 *Cedrusdeodara*nurseries. Dar and coworkers (2017) while working on root rot of *Abiespindrow*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 *Cedrusdeodara*in different nurseries

Nurseries showing root rot **Healthy nursery**

Table 1. Disease incidence of root rot in seedlings of *Cedrusdeodara*in 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**

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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**	

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 Table1 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*Phomaexigua*(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 *Phomaexigua*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 *Phomaexigua*(18.98%) and *Fusarium oxysporum*(7.49%), respectively. Isolation frequency of *Phomaexigua*and *Fusarium oxysporum*were significantly lesser as compared to *Rhizoctonia* spp. The results also revealed that population of Rhizoctoniawas 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 *Abiespindrow,* Dar *et al*. (2017) also revealed the presence of various pathogens *viz., Fusarium oxysporum*f.sp. *pini, Rhizoctonia*

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*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 *Cedrusdeodaran*seedlings

i) Post emergence damping off **ii)Drying and drooping down seedling symptom** **iii) Complete rotting of stem and drooping of seedling**

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

Range/ Nursery		Isolation frequency (%)												Mean	Pooled mean
		Fusarium spp.				*Rhizoctonia* spp.				*Phoma* spp.					
		2017		2018		2017		2018		2017		2018			
		Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root	Collar	Root		
Solan	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)**	
	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)**	**34.42 (32.81)**
	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 (14.06)**	**10.00 (17.58)**	**4.04 (9.92)**	**9.52 (17.38)**	**51.90 (46.17)**	**57.61 (49.82)**	**41.78 (38.28)**	**49.64 (43.06)**	**10.35 (12.94)**	**23.57 (27.03)**	**11.78 (13.36)**	**30.23 (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

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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 branced 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.2) identified as binucleate *Rhizoctonia* AG –E with a telemorphic stage *Ceratobasidium*sp. group CAG 6 and sequence was submitted to gene bank ((TomarM and Thakur R, 2020; Thakur R and TomarM. 2020).

Fig.2 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

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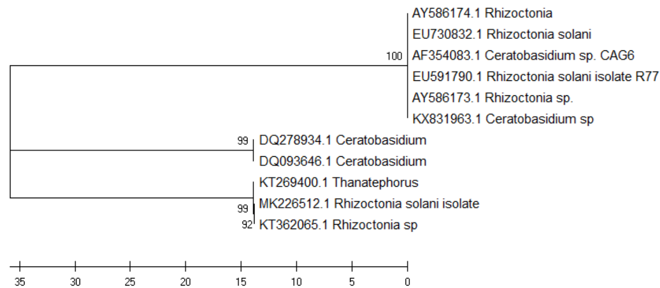
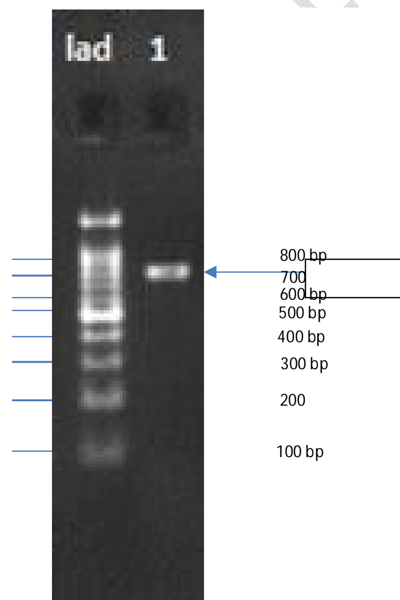


Plate 3. Agarose gel photograph of 706 bp amplicon of binucleate *Rhizoctonia* AG-E (Courtesy: Eurofins Genomics)



Pathogenicity test

The pathogenicity test of the isolated fungus was conducted on one month old rooted cuttings of *Cedrusdeodara* by standard Koch's postulates in nursery bags. Most frequently

isolated three main fungi *viz.,*binucleate *Rhizoctonia*AG-E, ~~*Fusarium*~~ *F.oxysporum*and~~*Phoma*~~ *P.exigua* were put to pathogenicity test to ascertain the causal organism of root rot in seedlings of ~~*Cedrus*~~ *C.deodara*. The results present in Table 2 revealed that maximum incidence (47.53%) was recorded in seedlings inoculated with binucleate *Rhizoctonia*AG-Efollowed by *Fusarium oxysporum*(3.55%) and *Phomaexigua*(0.00%), respectively. Morever, 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 ~~*Phoma*~~ *P.exigua*. ~~*Fusarium*~~ *F.oxysporum*produced wilting and needle chlorosis along with slight vascular discoloration after 35 days of inoculation (Plate 2) while ~~*Phoma*~~ *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 *Cedrusdeodara.* While working on stem blight of *Catharanthusrosea* (*V. rosea, Vinca minor*) and Vinca major (big-leaf or variegated) caused by the fungus *Phomaexigua, 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*, *Abiespindrow*and Urd bean (Rana and Tripathi,1983; Ahangar*et al*., 2011; Dar *et al*., 2013).**Plate 4, Table 3**

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Plate 4: Plates of Rhizoctonia culture.

Pure culture of *Rhizoctonia*Sclerotia formation in culture Monilioid cells of Rhizoctonia under compound microscope

Table 3. Per cent ~~Disease~~ disease ~~Incidence~~ incidence of root rot of *Cedrusdeodara* by three frequently isolated fungi under *in vitro* conditions

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Fungi	**Disease Incidence (%)**
Fusarium oxysporum	3.55
***Rhizoctonia* spp.**	47.53
Phomaexigua	0.00
CD0.05	1.21

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 3 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. 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 (Plate 3). 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

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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

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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 Nauni (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 ofthree 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*Phomaexigua*. 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 *Phomaexigua*(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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