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Research Article

Potential of Consortium of Native Microbiota Against Damping-off of *Cedrus deodara* Seedlings in North-Western Himalayan Region of India

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Abstract

Background and Objective: Soil-borne pathogens causing damping-off in the nursery can be managed by isolating and evaluating native biocontrol agents from the soil. The present study aimed at the isolation and preparation of a consortium of biocontrol agents against the damping-off pathogen. **Materials and Methods:** The native biocontrol agents were isolated from the soil, identified and purified. These native biocontrol agents were used against the damping-off pathogen *in vitro* and the four best biocontrol agents were selected for making a potent consortium against *Fusarium oxysporum* f. sp. *pini*. **Results:** *Trichoderma virens*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Aspergillus* sp., *Penicillium* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* were some of the microflora isolated and purified for testing against the pathogen. *In vitro* evaluation of the isolated biocontrol agents against the pathogen revealed that *Trichoderma virens*, *Trichoderma harzianum*, *Trichoderma viride* and *Penicillium* sp. were effective against the damping-off the pathogen. The consortium of four best fungal strains tested *in vitro* was prepared and inoculated in the nursery soil to test its efficacy *in vivo* against the damping-off of *Cedrus deodara*. *Trichoderma harzianum*+*Penicillium* sp.+*Trichoderma virens* showed maximum disease incidence reduction with only 29.44% incidence followed by integration of *Trichoderma viride*+*Trichoderma virens*+*Penicillium* sp. (31.33%) and *Trichoderma viride*+*Trichoderma harzianum*+*Penicillium* sp. (33.85%), respectively. **Conclusion:** The results highlight the potential of bio control agents present in the soil against the soil-borne pathogens causing severe diseases and hampering the production of healthy forests.

Key words: *Cedrus deodara*, nursery, damping-off, biocontrol agents, soil-borne

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Himalayas are a rich source of diverse flora and fauna. *Cedrus deodara* (Roxb.) G. Don, also known as the Himalayan Cedar is a species of the family Pinaceae and a primary evergreen tree species native to the Western Himalayas in India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh States and the Darjeeling Region of West Bengal), Southwestern Tibet, Western Nepal, Eastern Afghanistan and Northern Pakistan (mainly in Khyber Pakhtunkhwa) occurring at 1,500-3,200 m (4,921-10,499 ft.) altitude AMSL. Owing to its myriad properties, this tree is most valued in the region. Hence, the production of healthy seedlings becomes a prerequisite. Over few years major problems associated with this species in the region have been poor seed germination and fungal attack at the seedling stage (damping-off), resulting in high mortality. This led to poor germination and weak seedlings. Surveys in various nurseries of the state revealed poor germination of *Cedrus deodara* due to soil-borne diseases. Due to the onslaught of damping-off and root rot of the seedlings in the region, it became imperative to manage the attack of pathogens, especially through eco-friendly methods. Damping-off is a disease that leads to the decay of germinating seeds and young seedlings and is one of the most important yield constraints in forest nurseries. Over the years, numerous soil-borne fungi belonging to over a dozen genera viz., *Fusarium*, *Rhizoctonia*, *Macrophomina* and *Oomycete* (*Pythium* and *Phytophthora*) have been reported to cause damping-off on a large number of crops¹⁻³. Such pathogens caused severe damage to nursery plantations and most of the infected seedlings failed to survive or establish after their transplantation due to damping-off. Conventional fungicides are widely used to manage this disease but with major consequences. Whereas, on the one hand, fungicide overuse threatens human and plant health and causes ecological concerns, on the other hand, this has led to the emergence of pesticide-resistant microorganisms in the environment⁴⁻⁷. The application of biocontrol agents is an important substitute to conventional fungicides as it has lower negative impacts⁸. Managing plant pathogens by biological means through enhanced suppression of pathogen development in plants is in itself an actual green approach and co-inoculation of native biocontrol agents can be an effective strategy to manage the soil-borne pathogens in an eco-friendly way. Since scanty information is available on the use of consortium to evade damping-off in *Cedrus deodara*, hence, the objective of the present study was to sequester and identify native microbes from the soil and prepare a viable and effective consortium for use against

soil-borne diseases. The indiscreet use of chemicals has been ruining the soil microflora, therefore this study shall pave a way to redesign our strategies for healthy nursery production and help in utilizing the rich microflora for disease management. Thus, the present study was envisaged to develop sustainable and durable damping-off management strategies that are less reliant on conventional fungicides.

MATERIALS AND METHODS

Collection, isolation and identification of the pathogen:

The pathogen was collected from the various forest nurseries of Solan District of Himachal Pradesh, India (30.9084°N, 77.0999°E) during March, 2019. Plant samples were collected from different forest nurseries to ascertain the pathogen(s) associated with the damping-off of *C. deodara*. In each sample, ten seedlings of *C. deodara* showing any disease symptom viz., necrosis, wilting, drooping, stunted growth and damping-off were randomly collected. All samples were taken back to the laboratory for further analysis and stored in paper bags at 4±2°C inside the refrigerator. Standard tissue isolation procedures were followed to isolate the pathogen from the infected seedlings. The infected roots, crown portion of seedling and infected seeds were surface sterilized with 1:1000 mercuric chloride (HgCl₂) solution and repeatedly washed with sterilized distilled water to remove the traces of mercury (if any) and then transferred to sterilized Petri dish containing Potato Dextrose Agar (PDA) and incubated at room temperature (27±1°C). The isolated fungi were purified by the hyphal tip method on the Petri plates containing PDA. Pure cultures were kept in the refrigerator at 4±2°C for further studies.

Sequestration and identification of native microbiota:

Soil samples from the rhizosphere of diseased seedlings, healthy seedlings and healthy mature *C. deodara* trees in the wilderness from all the forest nurseries were collected for isolation of native biocontrol agents and sealed inside plastic bags and brought back to the laboratory for further analysis. All the samples were air-dried for 2-3 days in the laboratory at 23°C. The air-dried soil samples were finely ground using pestle and mortar, sieved with a 0.5 mm sieve to generate soil particles of a consistent size. The microflora viz., bacteria and fungi from the collected soil samples were isolated by following serial dilution plate technique⁹. The isolated organisms were further purified using the hyphal tip method and identified by examining the morphological characteristics in pure culture. The observations were compared with the standard authentic descriptions and taxonomic keys.

In vitro testing of selected microflora against the damping-off pathogen of *C. deodara*: The microflora was tested *in vitro* by dual culture method against the isolated pathogen in a completely randomized design. The data on diametric growth was recorded to assess the mycelial (%) inhibition in all the treatments, replicated 4 times. The mycelial (%) inhibition was calculated as per the formula given by Mwebaze and Owomugisha¹⁰:

$$I = \frac{(C-T)}{C} \times 100$$

Where:

I = Inhibition percentage

C = Colony diameter in control

T = Colony diameter in treatment

The microflora which resulted *in vitro* growth inhibition of the damping-off pathogen was selected and kept in the refrigerator for further studies.

Compatibility test among microbial agents: The selected microbial agents were tested for their compatibility with each other *in vitro* by dual culture technique. To carry out the consortium studies compatibility of selected microorganisms is necessary because incompatibility of the co-inoculants can inhibit each other as well as the target pathogen(s). The inhibition among them was examined and compatible biocontrol agents were further selected for the *in vivo* studies of consortium mediated defence of damping-off in *C. deodara* seedlings.

Preparation of consortium of best antagonists and *in vivo* testing against the isolated damping-off pathogen of *C. deodara*: The best four antagonists selected from the previous experiment were mass cultured on grain media (wheat-saw dust) and integrated in various combinations to form a practical consortium against the damping-off pathogen of *Cedrus deodara*. The pathogen was simultaneously mass cultured on maize grain-sand media. The experiment was conducted in a nursery polybag in completely randomised design. The mass culture of the pathogen along with the mass culture of various antagonists as per the treatments designed was added to the nursery polybag, a week earlier before sowing the seeds. Eleven such treatments were designed and replicated thrice to test their efficacy against the damping-off pathogen of *C. deodara*. The

observation on pre-emergence and post-emergence damping-off was recorded at 15 and 25 days after sowing, respectively:

$$\text{Pre/Post-Emergence damping-off} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants observed}} \times 100$$

Statistical analysis: The data recorded in each experiment were subjected to statistical analysis wherever required using MS-excel and OPSTAT¹¹. The differences exhibited by treatments were tested for their significance by employing a Completely Randomized Design (CRD)^{12,13}.

RESULTS AND DISCUSSION

Sequestration and identification of pathogen and biocontrol agents: The fungal pathogen(s) were isolated from infected seedlings collected during the survey of different forest nurseries in district Solan. In preliminary microscopic and morphological examinations, the isolated *Fusarium* strains were identified as *Fusarium oxysporum* f. sp. *Pini*¹⁴. The standard Koch's postulates were followed to prove the pathogenicity of the isolated strain of *Fusarium*. Based on the morphological characters, i.e., diameter and septation of hyphae, shape and size of spores and cultural characters, i.e., type and colour of colonies and pigmentation of culture were compared with standard authentic description and taxonomic keys given by various workers¹⁵⁻¹⁸. The fungus was identified as *Fusarium oxysporum* f. sp. *Pini* in Fig. 1. The results of identification were authenticated by the identification report from the National Centre of Fungal Taxonomy, New Delhi.

Numerous microorganisms and biocontrol agents were isolated from the soil samples by following the standard serial dilution technique. Out of numerous microorganisms isolated in Fig. 2, eleven fungal isolates were selected, purified and identified. The isolated and purified native biocontrol agents were identified by standard authentic descriptions and taxonomic keys¹⁵⁻¹⁸. These were used for further studies.

***In vitro* testing of native biocontrol agents against the damping-off pathogen of *C. deodara*:** The native biocontrol agents were tested *in vitro* against the damping-off pathogen of *C. deodara* seedlings i.e., *Fusarium oxysporum* f. sp. *pini*, by following the dual-culture technique and streak plate technique for fungal and bacterial isolates, respectively in Fig. 3. Table 1 indicates that, all the native biocontrol agents evaluated under *in vitro* conditions inhibited the growth of

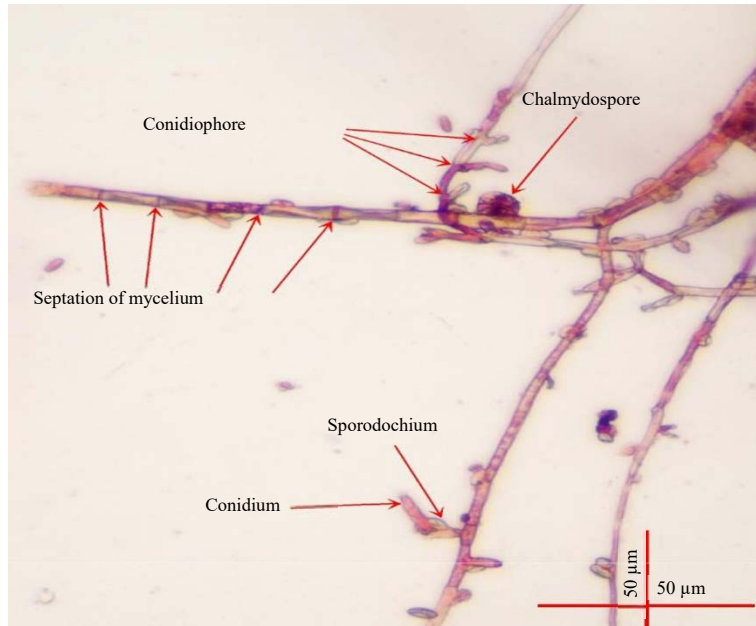


Fig. 1: *Fusarium oxysporum* f. sp. *pini* under 40X magnification



Fig. 2: Serial dilution of the soil samples

the damping-off pathogen. Out of the eight fungal isolates *Trichoderma*, *virens* was found to be the most effective against the damping-off pathogen, with mycelial growth inhibition of 83.61%, followed by, *Trichoderma harzianum*, *Trichoderma viride* and *Penicillium* sp., with 76.11, 74.99 and 68.61% mycelial growth inhibition, respectively. Among the two bacterial isolates, *Bacillus subtilis* was found to be better than *Pseudomonas fluorescence* with mycelial growth inhibition of 52.22% as compared to the *Pseudomonas* sp. with 51.72% mycelial growth inhibition.

Compatibility test among the native biocontrol agents: The native biocontrol agents that performed best against the damping-off pathogen viz., *Trichoderma virens*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Penicillium* sp., *Pseudomonas* sp. and *Bacillus* sp., were tested for compatibility amongst each other. The compatibility test was conducted by following dual-culture and streak plate techniques for fungal and bacterial isolates, respectively. The results of these *in vitro* experiments for judging the compatibility reaction among the native biocontrol agents are

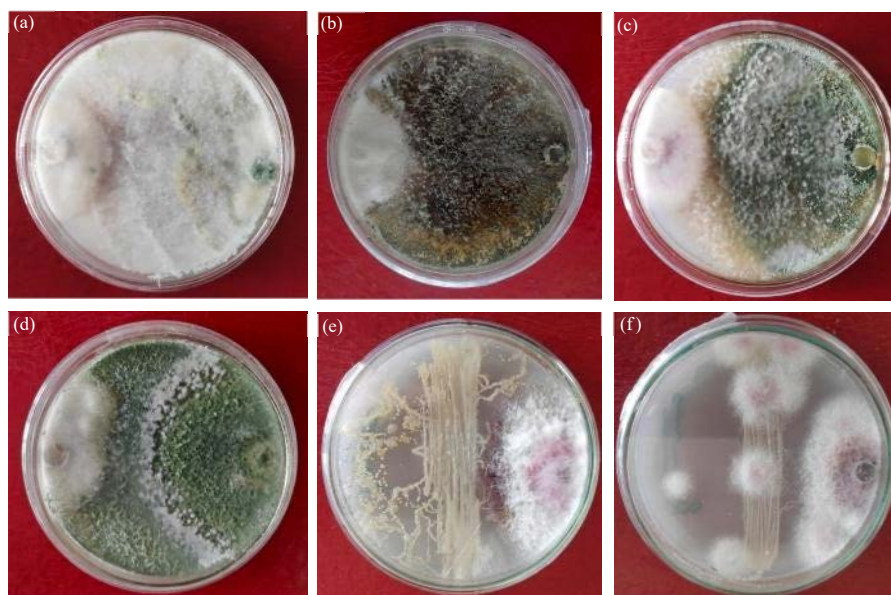


Fig.3(a-f): *In vitro* evaluation of isolated microbiota against *Fusarium oxysporum* f. sp., *pini*, (a) *Trichoderma virens*, (b) *Trichoderma harzianum*, (c) *Trichoderma viride*, (d) *Trichoderma hamatum*, (e) *Bacillus* sp. and (f) *Pseudomonas* sp.

Table 1: Mycelial growth inhibition of *Fusarium oxysporum* f. sp. *pini* by biocontrol agents under *in vitro* conditions

Biocontrol agents	Percent inhibition in mycelial growth
<i>Trichoderma virens</i>	83.61 (66.10)
<i>Trichoderma viride</i>	74.99 (59.99)
<i>Trichoderma harzianum</i>	76.11 (60.73)
<i>Trichoderma hamatum</i>	60.28 (50.91)
<i>Penicillium</i> sp.	68.61 (55.90)
<i>Botrytis</i> sp.	33.88 (35.58)
<i>Fusarium solani</i>	28.72 (32.39)
<i>Rhizoctonia</i> sp.	41.72 (40.21)
<i>Aspergillus</i> sp.	45.00 (42.11)
<i>Bacillus</i> sp.	52.22 (46.25)
<i>Pseudomonas fluorescens</i>	51.72 (45.96)
Control	0.00 (0.00)
C.D.	1.33

*Figures in parentheses are angular transformed values

presented in Table 2. Table 2 vividly shows that, all the biocontrol agents inhibited each other's mycelial growth. Amongst all biocontrol agents tested against each other, the interaction between *Trichoderma viride* × *Penicillium* sp., showed the least mycelial growth inhibition of 13.81%, closely followed by the interaction between *Trichoderma harzianum* × *Penicillium* sp. (22.22%), *Trichoderma virens* × *Penicillium* sp. (22.70%) and *Trichoderma hamatum* × *Penicillium* sp. (22.70%), respectively. The interaction between all the fungal isolates was found to be less than forty per cent. Whereas, the interaction among the fungal and bacterial isolates showed higher percentage of mycelial inhibition. Interaction of *Penicillium* sp. × *Bacillus subtilis* and *Penicillium* sp. × *Pseudomonas fluorescens*

showed 59.33 and 55.81% mycelial inhibition, respectively. Both the bacterial isolates i.e., *Bacillus subtilis* and *Pseudomonas fluorescens* produced a zone of inhibition and curtailed the growth of fungal biocontrol agents. Further, *in vitro* experiments to judge the compatibility of these native biocontrol agents by multiple-culturing them with each other were also carried out to closely examine the compatibility in a consortium. These results form the basis of the consortium based plant disease management strategy, as the growth of microorganisms that are to be used as soil inoculants against virulent races of soil-borne pathogens, itself must not get suppressed by the growth of the native soil microbial population.

***In vivo* testing of the consortium of native biocontrol agents against the damping-off pathogen of *C. deodara*:**

The pathogen of damping-off was mass cultured on maize-sand medium (1:1) and was mixed with sterilized soil in nursery polybags. The four best biocontrol agents viz., *Trichoderma virens*, *Trichoderma viride*, *Trichoderma harzianum* and *Penicillium* sp., were mass cultured on wheat grain-sawdust (1:1) medium separately and used as consortium against the damping-off pathogen *Fusarium oxysporum* f. sp. *pini* of *Cedrus deodara*. Completely Randomised Design (CRD) was used in the experiment. The pre-emergence and post-emergence damping-off data for each treatment was calculated at 15 and 25 days intervals. It is evident from the data presented in Table 3 that all the

Table 2: Mycelial growth inhibition and compatibility reaction among the native biocontrol agents under *in vitro* conditions

Biocontrol agents	Mycelial inhibition (%)	Compatibility reaction**
<i>Trichoderma virens</i> × <i>Trichoderma viride</i>	23.89 (29.61)	+
<i>Trichoderma virens</i> × <i>Trichoderma harzianum</i>	27.58 (31.67)	+
<i>Trichoderma virens</i> × <i>Trichoderma hamatum</i>	29.99 (33.19)	+
<i>Trichoderma virens</i> × <i>Penicillium</i> sp.	22.70 (28.44)	+
<i>Trichoderma virens</i> × <i>Pseudomonas fluorescens</i>	51.66 (45.94)	-
<i>Trichoderma virens</i> × <i>Bacillus subtilis</i>	49.18 (44.51)	-
<i>Trichoderma viride</i> × <i>Trichoderma harzianum</i>	28.73 (32.40)	+
<i>Trichoderma viride</i> × <i>Trichoderma hamatum</i>	26.77 (31.15)	+
<i>Trichoderma viride</i> × <i>Penicillium</i> sp.	13.81 (21.80)	+
<i>Trichoderma viride</i> × <i>Pseudomonas fluorescens</i>	49.22 (44.53)	-
<i>Trichoderma viride</i> × <i>Bacillus subtilis</i>	51.82 (46.02)	-
<i>Trichoderma harzianum</i> × <i>Trichoderma hamatum</i>	31.18 (33.93)	+
<i>Trichoderma harzianum</i> × <i>Penicillium</i> sp.	22.22 (28.10)	+
<i>Trichoderma harzianum</i> × <i>Pseudomonas fluorescens</i>	43.99 (41.53)	-
<i>Trichoderma harzianum</i> × <i>Bacillus subtilis</i>	47.25 (43.41)	-
<i>Trichoderma hamatum</i> × <i>Penicillium</i> sp.	23.22 (28.80)	+
<i>Trichoderma hamatum</i> × <i>Pseudomonas fluorescens</i>	41.33 (39.99)	-
<i>Trichoderma hamatum</i> × <i>Bacillus subtilis</i>	44.11 (41.60)	-
<i>Penicillium</i> sp. × <i>Pseudomonas fluorescens</i>	55.81 (48.32)	-
<i>Penicillium</i> sp. × <i>Bacillus subtilis</i>	59.33 (50.36)	-
<i>Pseudomonas fluorescens</i> × <i>Bacillus subtilis</i>	52.10 (46.19)	-

CD_(0.05) = 0.919, **Compatibility reaction: Compatible(+) and Incompatible(-), *Figures in parentheses are angular transformed values

Table 3: Effect of the consortium of native biocontrol agents on disease incidence of damping-off of *Cedrus deodara* seedlings under *in vivo* conditions

Treatments	Disease incidence (%)		
	15 days	25 days	Mean
<i>Trichoderma virens</i> + <i>Trichoderma viride</i>	44.18 (41.66)	63.22 (52.67)	53.70 (47.17)
<i>Trichoderma virens</i> + <i>Trichoderma harzianum</i>	41.92 (40.35)	58.92 (50.14)	50.42 (45.25)
<i>Trichoderma virens</i> + <i>Penicillium</i> sp.	37.55 (37.79)	56.29 (48.62)	46.92 (43.21)
<i>Trichoderma viride</i> + <i>Trichoderma harzianum</i>	42.85 (40.89)	58.03 (49.62)	50.44 (45.26)
<i>Trichoderma viride</i> + <i>Penicillium</i> sp.	39.18 (38.75)	51.00 (45.57)	45.09 (42.16)
<i>Trichoderma harzianum</i> + <i>Penicillium</i> sp.	33.88 (35.60)	47.66 (43.66)	40.77 (39.63)
<i>Trichoderma virens</i> + <i>Trichoderma viride</i> + <i>Trichoderma harzianum</i>	28.33 (32.16)	45.44 (42.38)	36.89 (37.27)
<i>Trichoderma viride</i> + <i>Trichoderma harzianum</i> + <i>Penicillium</i> sp.	25.88 (30.58)	41.81 (40.29)	33.85 (35.44)
<i>Trichoderma harzianum</i> + <i>Penicillium</i> sp. + <i>Trichoderma virens</i>	22.15 (28.07)	36.73 (37.31)	29.44 (32.69)
<i>Trichoderma viride</i> + <i>Trichoderma virens</i> + <i>Penicillium</i> sp.	24.62 (29.75)	38.03 (38.08)	31.33 (33.92)
Control	80.88 (64.08)	100 (90.00)	90.44 (77.04)
Mean	38.31 (38.15)	54.28 (48.94)	

CD_(0.05): Treatments = 4.03, Days = 1.72, Treatments × Days = 5.71, *Figures in parentheses are angular transformed values

treatments in consortia significantly reduced the incidence of damping-off of *C. deodara* seedlings over control. The integration of *Trichoderma harzianum*+*Penicillium* sp.+ *Trichoderma virens* resulted in maximum reduction of the disease incidence with only 29.44% incidence followed by *Trichoderma viride*+*Trichoderma virens*+*Penicillium* sp. (31.33%) and *Trichoderma viride*+*Trichoderma harzianum*+*Penicillium* sp. (33.85%), respectively. The disease incidence was significantly higher at 25 days (54.28%) as compared to 15 days (38.31%) after the sowing of seeds. The data also signifies the importance of combining three biocontrol agents over two as the maximum reduction in disease incidence was found in treatments where three bio control agents were used in consortia.

Cedrus deodara is important to forest tree species of the Himalayas. The nursery plantation, however, is threatened by many soil-borne pathogens viz., *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia* and *Macrophomina* etc. Therefore, the present studies were undertaken with the objectives to isolate and identify the pathogen associated with this disease and to devise a biological disease management strategy through the use of native microbial consortia against the damping-off pathogen under *in vivo* conditions. The symptoms of damping-off of *Cedrus deodara* seedlings were prominent in two stages as pre-emergence and post-emergence damping-off of the seedling. In pre-emergence damping-off, initial seed rot was predominant, causing non-emergence of the seedling above ground level

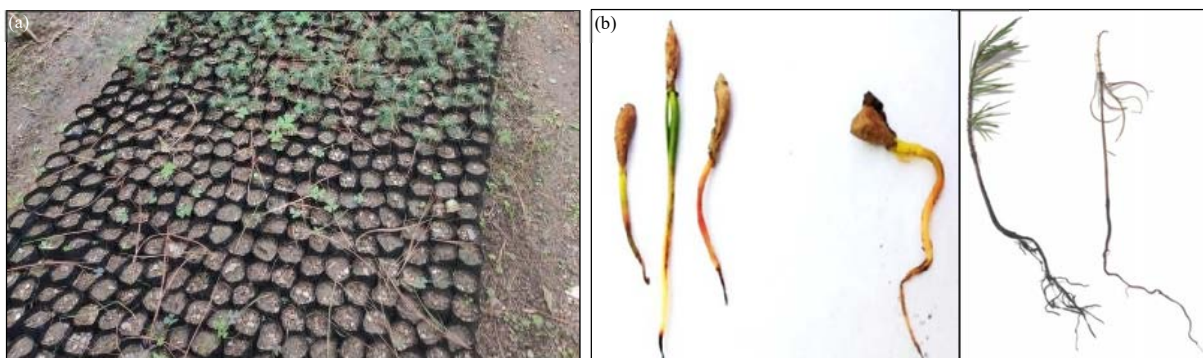


Fig.4(a-b): Symptoms of damping-off of *Cedrus deodara* in forest nursery, (a) Diseased nursery of *Cedrus deodara* and (b) Symptoms of damping-off in seedlings

and the affected seed showed low or no germination with blackening of plumule in later cases. In post-emergence damping-off, the stress due to the pathogen infection caused seedlings to dry up and wilt in the early stages of growth after germination. Severely affected seedlings suffered seedling rot, seedling mortality and late damping-off in Fig. 4a and b. Similar symptoms were observed by various workers on forest, vegetable and fruit nursery crops¹⁹⁻²². The morphological and cultural examinations of the damping-off pathogen *Fusarium oxysporum* f. sp. *pini* was done to ascertain its identity. The results of the identity of the causal organism are corroborated by the findings of various workers working on Mason pine, Fir, *Pinus patula* and Pines²³⁻²⁶.

The native microbiota present in the soil and plant roots was isolated in the present study, purified and tested against the damping-off pathogen. *Trichoderma virens*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Aspergillus* sp., *Penicillium* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* were some of the microflora isolated and purified for testing against the pathogen). Reports of isolation of *Trichoderma virens*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Aspergillus* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* from the rhizosphere of *C. deodara* from Solan district of Himachal Pradesh, India have been documented²⁷. The presence of chitinase enzyme in the microflora, phosphate solubilisation properties of bacteria, phytohormone production, protease and hydro cyanide production are some of the properties of the microflora making them efficacious against *Fusarium* species attacking various crops²⁸⁻³⁰.

In vitro evaluation of the isolated biocontrol agents against the pathogen revealed that *Trichoderma virens*, *Trichoderma harzianum*, *Trichoderma viride* and *Penicillium* sp., were effective against the damping-off pathogen. The present results align with the work done by

various workers who reported the affectivity of various *Trichoderma* species against soil-borne pathogens especially *Fusarium oxysporum*³¹⁻³⁴. The affectivity of *T. harzianum*, *Trichoderma viride* and *lacaria lacata* against *Fusarium oxysporum* f. sp. *pini* in Western Himalayan Fir (*Abies pindrow*) has been found to reduce damping-off infection²⁴.

To carry out the consortium studies, compatibility of selected microorganisms is necessary because incompatibility of the co-inoculants can inhibit each other as well as the target pathogen(s). In the present study, the fungal strains were inhibited by isolated bacterial strains. Therefore to make a potent consortium only the best fungal strains were chosen. The results agree with the various workers who reported the incompatibility of *Trichoderma* strains with *Bacillus* and *Pseudomonas* strains^{35,36}.

The consortium of four best fungal strains tested *in vitro* was prepared and inoculated in the nursery soil to test its efficacy *in vivo* against the damping-off of *Cedrus deodara*. The consortium was prepared by mixing the fungal isolates in different combinations. The pre-emergence and post-emergence damping-off data for each treatment was calculated at 15 and 25 days intervals. It was evident from the data that all the treatments in consortia significantly reduced the incidence of damping-off of *C. deodara* seedlings over control. The integration of *Penicillium* sp.+*Trichoderma virens*+*Trichoderma harzianum* resulted in the least disease incidence (29.44%) followed by *Penicillium* sp.+*Trichoderma viride*+*Trichoderma virens* (31.33%) and *Trichoderma harzianum*+*Penicillium* sp.+*Trichoderma viride* (33.85%), respectively.

Results are in agreement with other studies by various workers. Similarly, the consortium of *Trichoderma harzianum*+*Pseudomonas fluorescens*+*Bacillus subtilis*+*Rhizobium* sp., showed remarkable disease reduction against soil-borne pathogens of chickpea, followed by the consortium

of *Trichoderma harzianum*+*Bacillus subtilis* and *Pseudomonas fluorescens*+*Rhizobium* sp. (77%), respectively. While working on *Fusarium* wilt of Banana using a consortium of several endophytes viz., *Trichoderma* sp. strains and *Pseudomonas* sp. Strains there was 79% decrease in the incidence of the disease, as compared to the mono application of this biocontrol agents³⁷⁻⁴⁰.

CONCLUSION

The damping-off of *Cedrus deodara* is a serious threat to healthy forest nurseries. The weak and diseased seedlings give rise to poor stand and hence lead to huge losses. The isolation of potent biocontrol agents and preparation of a potent consortium was found to be beneficial and effective in managing damping-off disease caused by *Fusarium oxysporum* f. sp. *pini* in *Cedrus deodara* seedlings. This study can prove to be a baseline for developing the potent consortium and commercializing it for the benefit of the growers.

SIGNIFICANCE STATEMENTS

This study discovered the potential of native microflora against deadly soil-borne pathogens such as *Fusarium*. A healthy nursery is the backbone of a healthy tree. This study will help the researchers to uncover the critical areas of untapped forest wealth in terms of biocontrol agents that can prove to be an eco-compatible and sustainable way of raising healthy forest nurseries and help to mitigate the delirious effects of unabashed use of pesticides, thus reducing the carbon footprints on the planet earth.

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