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Cedrus deodara root rot disease-threat to the Himalayan forestry and environment

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ABSTRACT: Studies on root rot disease of deodar *(Cedrus deodara)* caused by pathogen *Phytophthora cinnamomi* are being reported for the first time from Himalayan region. Disease resulted in wide spread mortality of deodar trees near Chail, Shimla. About 200 trees have completely dried up, and another 150-200 are in different stages of withering. Whereas the grown up trees face this disease problem, the young regenerating plants are healthy in spite of ample inoculum in the soil. Extensive ectomycorrhizal presence in such plants acts as the main deterrent to invasion by pathogen. In older trees, where ectomycorrhizal mantle is lost from short roots, the surface gets exposed to attack by invading pathogen. The pathogen produces zoosporangia in the soil extract each releasing 30-40 kidney shaped zoospores. This is the first report of any fungus causing such a large-scale disease of forest trees in Indian Himalayas.

Key words: *Cedrus deodara,* root rot, *Phytophthora*

Cedrus deodara, the state tree of Himachal Pradesh is one of the most important conifers in the Himalayan moist temperate forests. It extends from Afghanistan through the hilly regions of Pakistan and India to Kurnauli valley in Nepal occupying an elevation of 1200-3050 m. The total area of deodar forests in India is estimated to be around 203,263 hectares. Out of this, 69,872 hectares lie in Himachal Pradesh (Anon, 1976). Deodar forests play an important role in the economy of the country, as its wood is the strongest and most durable. Further, the elegant trees of *C. deodara* add to the scenic beauty of the Himalayas. Disease aspects of this valuable tree till date are subjected to only scant studies. A search into literature reveals that only a few diseases alongwith causal organisms were listed and described by Maheshwari and Biswas (1970) and Bakshi (1976). Hence, it becomes difficult to decide if any unreported disease condition is observed and whether it is caused by a native or an introduced pathogen. Introduced diseases, when allowed to run unchecked, can affect the aesthetic and thus the recreational value of the forest, which is of prime importance in the Himalayan region, in general, and Himachal Pradesh in particular. Schematic presentation of the infected forest is given in Fig.1.

This paper, for the first time, reports studies on root rot of *C.deodara* caused by *Phytophthora cinnamomi* in Chail forest of Himachal Pradesh where vast stretch of deodar trees was observed to undergo unnatural drying. The fungus caused serious damage, devastating around 7-8 ha of pure deodar forest posing challenge to the economy, ecology and environment of the area.

MATERIALS AND METHODS

Site description

The affected deodar forest is located about 2-2.5 km, from famous tourist destination Chail in the outskirts of Shimla City at about 1950 metres above MSL. Deodar is the dominant species in the entire forest and forms almost pure stands. The affected forest on the first look appeared as if it has been destroyed by fire (Fig 2a). Almost all the mature trees in the area extending to about 5-7 hectares were dried, and died and others are standing in various stages of decay. Whereas, some of the dead and dry standing trees still have the branches and bark intact, the advanced stages of decay are marked by gradual shedding of branches and bark. In some of the decayed trees, the exposed wood beneath the bark bears lesions indicating that trees must have been dead for quite some years. Local people corroborate these observations stating that the decay process started about 18-20 years back.

Sample collection

In the infected forest trees were found in different stages of disease development. The infected trees were

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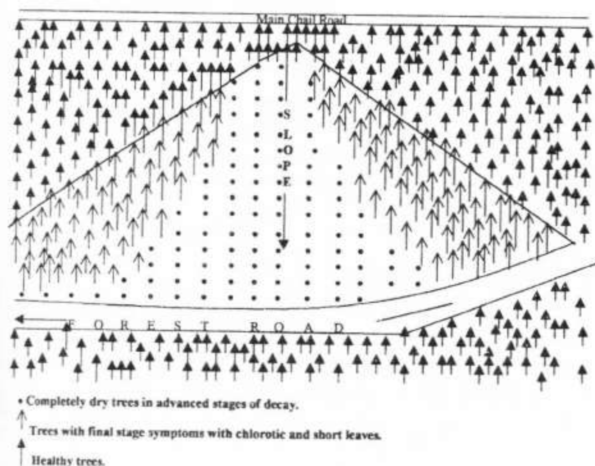

Fig.1. Schematic presentation *Cedrus deodara* forest infected with *Phytophthora cinnamoni*

grouped into eight categories: (i) debarked in advanced stages of decay, (ii) dried and dead trees with some bark intact and still to undergo decay, (iii) dried and dead trees with bark and branches intact but without needles, (iv) dried and dead trees with bark, branches and dry needles intact, (v) dried and dead trees with bark, branches and needles intact but defoliation of needles has just started at the ends of branches, (vi) living trees with chlorotic foliage and no defoliation yet, (vii) trees without any visible symptoms of disease and (viii) young regenerating healthy plants. Soil, root and wood samples of all eight categories were collected in autoclaved polypropylene bags in triplicate.

Fungal isolation

For isolation from diseased roots, wood, bark and soil, potato dextrose agar (PDA) medium was used. The infected root pieces were washed briefly in water, dipped for a few seconds in 70% alcohol, blotted on a pre-sterilized Whatman filter paper and placed on PDA in petriplates and slants in test tubes. For isolation from wood and bark, a shallow cut was given on the surface of wood and bark and then these were broken into pieces by applying pressure with fingers along the cut exposing the unexposed uncontaminated portion. From this freshly exposed portion, a piece of tissue was quickly lifted with forceps and placed on the medium in petriplate. Culture isolation was done directly from the soil crumbs and by soil dilution method on PDA having streptomycin sulphate to inhibit soil bacteria and fungi. Soil crumbs were directly sprinkled on the surface of petriplates containing PDA. Soil dilutions were prepared by mixing lg of soil in 100 ml of sterilized distilled water. For isolation on petriplates, 1ml of the solution from 1:4 dilution was used. Petriplates were incubated at 23°C for 72 hours under dark conditions.

Isolation of chlamydospore

Chlamydospores are released into the soil following decay of roots. Chlamydospores were recovered and recorded from soil by dilution plate method and direct plating of soil crumbs. Dilutions were prepared from the suspension of one g soil in 100 ml of sterilized distilled water.

Zoosporangial production

Zoosporangial production was tried in non-sterile soil leachate as described by Mehrlich (1935) with slight modifications. Colonies of cultures were produced on 100 ml potato dextrose broth (PDA-agar) from 8mm culture discs. After four days of growth, colonies were repeatedly washed in sterilized distilled water to remove the unutilized nutrients. Colonies were transferred to flask containing 100 ml unsterilized soil leachate prepared by mixing 10 g soil in 100 ml distilled water and filtering through Whatman No.41 filter paper. Flasks were incubated at 23°C

Morpho-anatomical details of root

Roots were washed in running tap water to remove the adhering soil particles. Roots were observed for morphological details under stereo microscope. For anatomical studies, roots were fixed in formalin acetic acid and 70 % alcohol (5:5:90) for 24 hours and stored in 70 % alcohol. Sectioning was done with hand using razor blade.

Pathogenicity test

Pathogenicity of the isolates was tested through Koch postulates. Healthy seedlings of *Cedrus deodara* were inoculated with the pathogen under glass house conditions. Mass inoculum of pathogen was prepared in two litre flasks containing one litre of potato dextrose broth. Flasks were inoculated with 8 mm discs of culture. Pieces of broken glass were placed in broth for the maceration of mycelium through constant stirring for 1-2 minutes every day. Flasks were incubated for one month in incubator at 25 ± 1°C. After one month medium was decanted off and macerated mycelial culture was thoroughly washed with sterilized distilled water 2-3 times to remove any unutilised nutrients. Mycelium harvested from three flasks was mixed in two litres of sterilized distilled water. *C deodara* seedlings were uprooted from the polythene bags and directly dipped in this mycelium slurry, slightly smeared

so that mycelium adheres to the roots. These were replanted in the polythene bags. Uprooted seedlings dipped in plain sterilised distilled water served as control.

RESULTS

Symptoms

The disease has resulted in the decline and death of trees above 15" diameter at breast height (DBH) and older trees. The foliage in the infected trees turns yellowish, the length of needles and shoot is reduced and finally the diseased trees become conspicuous with scant and chlorotic foliage extending in centripetal manner (top to base and from end of branches towards the stem). Cone size is also considerably reduced. The tops of the trees and tips of the branches dry up (Fig 2b). The photograph shows localised disease in a patch of deodar trees. Below the diseased patch runs the forest road and below this road all the trees are healthy. The road, therefore, seems to have checked the spread of the disease. In the backdrop of infected patch, naturally regenerating plants of *C. deodara* are clearly visible. All these are healthy plants. On investigation, all these were found to have well-developed ectomycorrhizal roots, which apparently deter infection by the pathogen.

Fungal isolation

The culture of *P. cinnamomi* isolated from the soil and infected roots of *C. deodara* is white in colour and the colonies exhibit characteristic rosette growth pattern (Fig. 2d). Fungus could be easily isolated from soil and roots from all samples except roots of completely dried trees showing advanced stages of decay and from roots of young regenerating plants. Even the soil from near the vicinity of the trees without visible symptoms had the same quantum of inoculum as that near the highly diseased trees. No inoculum was, however, present in the wood and bark of any category of trees in the diseased forest. Detail of fungal isolation from different parts of the trees, showing symptoms of disease development in different stages, is given in Table 1.

On microscopic examination, fungus has been found to possess two types of well-developed profusely branched, hyaline mycelia, which are coenocytic and moderately thick. Subterranean mycelium is composed of hyphae 5-6 µm in diameter. Cytoplasm contains scattered nuclei. Older hyphae occasionally undergo septation. The fungal hyphae ramify the intercellular spaces of the cells of host root tissue. Ageing cultures produce

Table 1. Isolation of *P. cinnamomi* from different parts of trees showing symptoms in different stages of development

Sample Category	Soil	Root	Bark	Wood
Debarked trees in advanced stages of decay	+	–	–	–
Dried and dead undecayed trees with some bark intact	+	+	–	–
Dried and dead trees with bark and branches intact but without needles	+	+	–	–
Dried and dead trees with bark, branches and dry needles intact	+	+	–	–
Dried and dead trees with bark, branches and needles intact but defoliation of needles has just started at the end of branches	+	+	–	–
Living trees with chlorotic foliage and no defoliation yet	+	+	–	–
Trees in the diseased patch without any visible symptoms of disease	+	+	–	–
Young regenerating healthy plants with mycorrhiza	+	–	–	–

\+ *P.cinnamomi* present ; - *P.cinnamomi* absent.

a number of chlamydospores on lateral hyphal branches. Chlamydospores are thin walled, globose 20-45 µm in diameter. Chlamydospores germinate through multiple germ tubes (5-7) on potato dextrose agar medium. No sex organs have been observed in culture, however.

Chlamydospore isolations

Isolations from soil made through dilution plate technique were directly observed under stereo microscope. Germinating chlamydospores were frequently observed in the samples. The chlamydospores in the petriplates were observed to be without any germination activity. The number of germinating chlamydospores was more in soil collected from recently dried trees and trees showing initial stages of disease development. The soil samples collected from the dry trees with advanced stages of decay exhibited comparatively less germination activity of the chlamydospores. No

Fig. 2. (a) Dead and dry trees of *C.deodara* in different stages of infection; (b) Patch of infected trees of *C.deodara* in the forest with chlorotic foliage; (c) *Cedrus deodara* roots showing fungus mycelium and dead short roots; (d) Isolated culture of *Phytophthora cinnamomi* showing characteristic rosette growth on PDA; (c) Healthy roots of regenerating seedlings with conspicuous ectomycorrhizal development; (f) T. S. of ectomycorrhizal root showing thick outer fungal sheath (mantle); (g) T. S. of uninfected short roots; (h) T. S. of diseased short root.

isolation of chlamydospores was achieved from the bark and wood samples of any category of trees.

Sporangial production

Sporangial production is more complicated in *P. cinnamomi* than other species of this genus. Sporangia of *P. cinnamomi* are non-papillate. They are generally ellipsoid to ovoid in shape, 20.0-35.5 µm wide × 55.0-75 µm long. Each sporangium produces 30-40 kidney shaped biflagellate zoospores, each 10- 12 µm in diameter.

Morpho-anatomical details of roots

Plate 1 e shows the morphology of mycorrhizal roots in a healthy regenerating plant of deodar. The ectomycorrhiza covers the roots entirely, is creamish

white in colour and is branched in a corralloid manner. In T. S., such roots reveal the presence of a 45-60 μm thick mantle (Fig. 2f) and well developed Hartig net (Fig. 2f). Fig. 2g is the T. S. of a short root, from an older infected tree; the root is yet to be infected and T. S. reveals the normal root anatomy but there is no hyphal mantle nor there is any Hartig net. Plate l h is the T. S. of an infected short root showing its fate after infection with the pathogen. The root is shrivelled, all the component tissues are deformed, there is lot of tannin deposition in the cortex and the vascular tissue shows lignification. Ultimately, the roots acquire charcoal black colour. Fig. 2c shows the profuse growth of the fungus under bark of the mother roots. The short roots are also infected. The fungus from mother roots and directly from the soil and adjacent infected roots extends to the lateral short roots and colonises the inter and intra-cellular spaces including vascular tissues.

Pathogenicity test

Pathogenicity test was performed following steps proposed by Koch (1876). *P. cinnamomi* culture was isolated from the roots of infected *C.deodara* trees. Pure culture was multiplied in PDA broth in flasks. It was compared with the original culture for purity. Seedlings inoculated with mass culture developed chlorotic leaves, shorter in length and which dried completely within six months of inoculation. Symptoms appeared at the onset of spring season. There was reduction in leaf size, chlorosis of leaves and absence of new shoots in the seedlings inoculated with pure culture of fungus as observed in the infected forest trees. Short roots in artificial inoculated seedlings were dark brown to black in colour resembling short root collected from infected forest trees. A comparison of reisolate with original isolate reveals them to have same characteristics conforming similarity of the two and proving the pathogenicity of the fungus.

DISCUSSION

The species of *Phytophthora* are well known pathogens of a wide range of hosts the world over. In India, so far *Phytophthora cinnamomi* had been reported only from Andhra Pradesh causing root rot of grapes (Agnihothrudu, 1968) and from stem and roots of Cincona from Anamalai, Tamil Nadu (Ramakrishnan, 1951). Other species of the genus *Phytophthora* have been reported to cause diseases of agricultural and horticultural crops. Infection of conifers in the Himalayas by any species of *Phytophthora,* is first ever report on deodar. *P. cinnamomi* was first described by Rands (1922). Now it is known from over 60 countries causing, infection of more than 900 host plants (Zentmyer, 1980). The same fungus had earlier earned notoriety for wiping out Jarrah tree *(Eucalyptus marginata)* in western Australia causing "jarrah die-back', It also causes short leaf disease of short leaf pine *(Pinus echineta)* in the United States. The fungus is now having repeat performance of notoriety earning the same bad name in mild temperate, subtropical and tropical areas by infecting woody plants.

In deodar, the infected deodar plants developed chlorotic foliage with considerable reduction in leaf size. This is essentially a reflection of nutrient deficiency which, results due to the death of short absorbing roots and poor regenerative capacity of the ageing main roots. Growth of *P. cinnamomi* was noticed to be more vigorous during rainy season when there is plenty of moisture and the temperature is optimum for the germination and movement of fungal propagules, which are motile zoospores. During infection, most of the short roots are infected and rendered dead, by the time next spring season sets in. Demand of plant for higher uptake of nutrients in the season is not fulfilled, which ultimately results in the abrupt appearance of chlorotic foliage and decline in the growth of trees. In seedling stage, death may occur within a few days of the appearance of symptoms but in mature trees, 15-20 years can lapse before they actually succumb. Pathogen can survive, and multiply saprophytically on dead organic matter. The disease can also spread from one tree to another by root contact or by zoospores in the presence of water,

In the area under study, the young regenerating plants of deodar were healthy and their roots were also not infected even though the soil where these were growing had enough inoculum of *P. cinnamomi.* Examination of such roots revealed that these roots were profusely ectomycorrhizal (Fig.2e) with thick hyphal mantle (Fig. 2f). This suggests that the ectomycorrhizal mantle was acting as a mechanical barrier, not allowing the entry of pathogen. The older trees were however, seriously infected. In older trees, short roots were without ectomycorrhizal sheath (Plate 1 g) and hence they were more prone to infection by the pathogen. It has also been reported that breakdown of mycorrhizal protection may also occur during periods of prolonged rainfall (Bassett and Will, 1964; Marks, 1965; Newhook, 1959) by bacterial lysis (Foster and Marks, 1967) and by the rupture of mycorrhizal mantle during root elongation (Marks and Foster, 1967) making the roots vulnerable to infection.

In some cases, mycorrhizal fungi and microorganisms harbouring the soil around short roots are reported

to produce some antifungal and antibacterial compounds, which inhibit root pathogenic infections (Marx, 1971). However, the significance of antibiotic production by saprophytes in reducing the inoculum potential of root pathogens and of subsequent root disease development is not yet fully understood.

Detailed studies related to specific interaction between the mycorrhizal fungi and *P.cinnamomi* on loblolly pine have been published by Marx and Davey (1969a, 1969b). These studies contain substantial evidence showing the role of ectomycorrhizae as biological deterrent to the infection of roots by pathogens, including *P. cinnamomi.* Complete absence of any apparent disease symptoms from the young regenerating deodar plants in the affected forest area and the presence of vigorously growing ectomycorrhizae on their roots are clear indications to the ectomycorrhizal association acting as physical barrier to the infection of these plants by the pathogen.

Another interesting feature of this study is that *P. cinnamomi* propagules (mainly chlamydospores) were detected in advance of symptom development. *P. cinnamomi* was isolated from the asymptomatic trees sampled in the forest adjacent to infected trees where no mortality had yet occurred. The regular cattle grazing and human trespassing observed during samplings in the infected forest may be one of the probable means of inoculum dissemination to the adjoining areas. This observation necessitates the need to collect soil samples beyond the areas of mortality to determine the extent of spread of the pathogen to optimize disease control procedures.

The wide host range of the pathogen and facultative mode of parasitism ensure the survival of this pathogen in one form or another, thus making control measures difficult. Localized fumigation of soil has been used to check the disease but this cannot be adopted for vast stretches of forest land. Pratt (1971) suggested the possibility of controlling *P. cinnamomi* by natural or induced antibiosis. Newhook and Podger (1972) have discussed in detail this approach for control on a large scale, but still a lot more research is needed to achieve the desired results. Marx (1973) concluded that in all probability most of the mechanisms of root protection of mycorrhizae are functional at any given time since several appears to be inseparable (i.e, mantle barriers, host origin inhibitors, differences in chemical exudation, etc.). This broad spectrum of defence mechanisms acting in concert assumes greater opportunities for biological control of root pathogen by mycorrhizae.

Keeping in view the reported severity of *P. cinnamomi* infection from other countries, it is important to take all precautions against the further spread of this deadly disease in the north-western Himalayas.

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REFERENCES

Agnihothrudu, V. (1968). A root rot of grapes in Andhra Pradesh. *Curr. Sci.* **37** : 292-294.

Anon, (1976). Indian Timber. Deodar Information Series-18 compiled at the Editorial Board. Forest Research Institute, Dehradun, pp 13.

Bakshi, B.K. (1976). *Forest Pathology: Principles and Practices in Forestry.* Forest Research Institute Press Dehradun. pp 400.

Bassett, C. and **Will, G. M.** (1964). Soil sterilisation trials in two forest nurseries. *New Zealand J. Forest.* **9** : 50-58.

Foster, R. C. and **Marks, G. C.** (1967). Observations on the mycorrhizas of forest trees 11, The rhizosphere *of Pinus radiata* D.Don. *Aust. J. Biol. Sci.* **20** : 915-926.

Koch, R. (1876). Die Actiologie der Milzbrand-Krankheit, begrundet auf die Entwick lungsgeschichte *des.Bacillus anthracis,* Beiter. *Biol. Pflanz* **2** : 277-310

Maheshwari, P. and **Biswas, C.** (1970). *Cedrus.* Council of Scientific and Industrial Research, New Delhi, pp 115.

Marks, G. C.(1965). The pathological histology of root rot associated with late damping-off in *Pinus lambretiana. Aust. Forester.* **29** : 30-38.

Marks, G. C and **Foster, R.C.** (1967). Succession of mycorrhizal associations on individual roots of radiata pine. *Aust. Forester* **31** : 193-201.

Marx, D.H. (1971). Ectomycorrhizae as biological deterrents to pathogenic root infections. In: *Mycorrhizae* (Ed., E. Hacskaylo), pp 80-96. U.S. Government Printing Office, Washington D.C.

Marx, D.H. (1973). Mycorrhizae and feeder root diseases. In: *Ectomycorrhizae* (Eds., Marks, G, C. and Kozlowski, T.T.), pp 351-382, Academic Press, New York.

Marx, D,H. and **Davey, C. B.** (1969a). Resistance of aseptically formed mycorrhizae to infection by *Phytophthora cinnamomi. Phytopathology,* **59** : 549-558,

Marx, D.H. and **Davey, C.B.**(1969b). Resistance of naturally occurring mycorrhizae to infection by *Phytophthora cinnamomi. Phytopathology.* **59** : 559-565.

Mehrlich, F.P. (1935). Non-sterile soil leachate stimulating to zoosporangia production by *Phytophthora* sp. *Phytopathology* **25** : 432-434.

Newhook, F.J. (1959). The association of *Phytophthora* sp, with mortality of *Pinus radiata* and other conifers 1. Symptoms and epidemiology in shelter belts. *New Zealand J. Agri. Res.* **2** : 808-843.

Newhook, F.J. and **Podger, F.D.** (1972). The role of *Phytophthora cinnamomi* in Australian and New Zealand forest. *Ann. Rev. Phytopathol.* 229-236.

Pratt, B.H. (1971). Isolation of basidiomycetes from Australian *Eucalyptus* forest and assessment of their antagonism to *Phytophthora cinnamomi. Trans. Brit. Mycol. Soc.* **56** : 243-251.

Ramakrishnan, T. S, (1951). Addition to fungi of Madras-X. *Proc. Ind. Acad. Sci.* **43B** : 63-72.

Rands, R. D. (1922). Streepkanker van kaneel, veroorzaakt door *Phytophthora cinnamomi* n. sp. (Stipe canker of cinnamon caused by *Phytophthora cinnamomi* n.sp.). *Meded. Inst. Plantenziekt* **54**: 41.

Zentmyer, G.A. (1980). *Phytophthora cinnamomi* and diseases it causes. Monograph No.10. *Amer. Phytopathol Soc.* St. Paul Min. 96 pp.

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