International Journal of Agricultural Science Research Vol. 4(7), pp.146-149, July 2015
Available online at http://academeresearchjournals.org/journal/ijasr
ISSN 2327-3321 ©2015 Academe Research Journals

Full Length Research Paper

Ectropis deodarae and fungal pathogen: A potential threat to *Cedrus deodara* in the Himalayan forestry

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Accepted 16 July, 2015

The affected deodar forest is located about 95 km (Habban) from Solan Himachal Pradesh, India where deodar is the dominant species in the entire forest and forms almost pure stands. More than 500 trees in 3 different sites were dried, and died and others are standing in various stages of decay. Defoliated patches of deodar tress were visible from long distance giving an unhealthy, brownish and burnt appearance from a long distance. The objectives of this study were to identify the different causes of defoliation of deodar forests. Among them major was insect damage caused by *Ectropis deodarae* (deodar defoliator), as well as mites which were also associated along with different fungal strains. Seven genera of fungi were also isolated from the infected forest of *Cedrus deodara*. These fungi were identified as *Phytophthora* sp., *Fusarium sp*., *Aspergillus sp.*, *Alternaria sp*., and *Trichoderma* species on the basis of morphological as well as microscopic examination. Among all the isolates, *Phytophthora* species was the most prevalent in all the samples collected. Therefore further study was conducted on the pathogenicity of the *Phytophthora* on the different plants leaves, which resulted in the total defoliation of the leaves. Furthermore, *Trichoderma* sp. was observed as the most effective antagonists against the *Phytophthora*. So, potent biocontrol agents against pathogens infecting *C. deodara* are the need of the hour.

Key words: *Cedrus deodara*, *Ectropis deodarae*, fungus, himalayas, pathogenicity.

INTRODUCTION

Cedrus deodara is one of the real cedars. It is in the Pinaceae family which includes both evergreen and deciduous conifers like pine trees, spruce trees and fir trees. The true cedars consist of 4, or fewer, closely related species of tall, oleoresin-rich, monoecious, coniferous, evergreen trees, with geographically separated distributions (Maheshwari and Biswas, 1970; Farjon, 1990; Vidakovic, 1991; Tewari, 1994). The cedars are restricted to the mountain or high montane zones of mountains, situated roughly between 15°W and 80°E and 30° to 40°N (Farjon, 1990). This discontinuous range is composed of 3 widely separated regions in North Africa and Asia: 1) the Atlas Mountains of North Africa, in northern Morocco and northern Algeria; 2) Turkey, the mountains on Cyprus and along the eastern border of the Mediterranean Sea in Syria, Lebanon; and 3) the Hindu Kush, Karakoram and Indian Himalayas. The 4 species of *Cedrus* namely, *Cedrus atlantica*, *Cedrus brevifolia*, *Cedrus deodara* and *Cedrus libani* are so closely related due to the fact that the habitual characteristics help to differentiate the species (Farjon, 1990).

Deodar cedar will usually be 40-70' tall and 20-40' wide, forming a pyramidal shape. It can be almost 200' tall in its native habitat. Each needle on this conifer is 1-2" long and can be bluish-green or grayish-green depending on the cultivar. They are produced in whorls of 20-30 needles. They also appear individually on long shoots. This is a monoecious species that will have both male and female flowers and cones present on the same tree. The fruit is a cone that will be reddish-brown and 3-4" long at maturity. It will eventually break apart to allow the seeds to disperse (Maheshwari and Biswas, 1970; Vidakovic, 1991).

Deodar forest play an important role in the economy of

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the country, as its wood is the strongest and most durable. Further, it is in great demand as building material because of its durability, rot-resistant character and fine, close grain, which is capable of taking a high polish. Its historical use to construct religious temples and in landscaping around temples is well recorded. Its rot-resistant character also makes it an ideal wood for constructing the well-known houseboats of Srinagar, Kashmir. Cedar oil is often used for its aromatic properties, especially in aromatherapy. It has a characteristic woody odor which may change somewhat in the course of drying out. The crude oils are often yellowish or darker in color. Its applications cover soap perfumes, household sprays, floor polishes and insecticides (Singh and Agarwal, 1988). 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 along with causal organisms were listed and described by Maheshwari and Biswas (1970) and Bakshi (1976). Recently we have observed the drying of Deodar plants, so we visited the site of infected plants and collected the soil, root, bark and wood samples as well as larvae and pupae of *Ectropis deodarae*. In the present study, we reported *C. deodara* associated fungal communities as well as insect pest *E. deodarae* from Himalayan forests. The present study is significant in the identification of some biocontrol agents against pests and pathogens, thus protecting a valuable asset of nature (*C. deodara*), from them.

MATERIALS AND METHODS

Site description

The affected deodar forest (Habban) is located about 35 km, from Rajgarh District Sirmour, Himachal Pradesh. 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. More than 500 trees in 3 different sites 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. The forest rangers and guards corroborate these observations stating that the decay process started about 3-4 months back (March to June).

Sample collection

The samples of infested deodar, larval and pupae stages of the pest were collected. The plastic container containing deodar fresh branches were used for larvae collections, whereas moistened soil with pupae stages were collected separately and brought to lab for further investigation.

The different samples from rhizosphere soil and non rhizosphere soil, root, bark and wood were collected separately from two different sites in the ziplock polypropylene bags in triplicate for fungus isolation.

Insect identification and fungal isolation

The caterpillar was identified by visual examination as well as under stereomicroscope. The fungi were isolated from different soil samples on the 2% Malt extract medium (MEA) with pH 5.8-6.0. The soil samples were serially diluted (by taking 1 g soil and 9 ml distilled water) aseptically and then spread on malt extract plate. The infected root samples were washed properly in water, dipped for a few seconds in 70% alcohol, blotted on a pre-sterilized Whatman filter paper and placed in the malt extract agar petriplates. For isolation of fungi 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 forceps and a piece of tissue was quickly lifted with forceps and placed on the malt extract plate. After that all the plates were incubated at 25°C for 5 days.

Morphological identification of fungi

After 5 days of growth of all the samples, the fungal cultures were identified on the basis of colony morphology as well as microscopic examination. The slides were prepared by taking loopful of mycelium and mixed with Lacto phenol blue and by placing cover slip over it. The mycelium was teased properly with the help of the backside of a pencil by taking care of cover slip so that it should not broke down and was visualized under microscope at 10×, 40× and 100×.

Pathogenicity test

Pathogenicity of the isolates was tested through Koch postulates. In the laboratory experimentation, the leaves bioassay were conducted on 3 different plants leaves and all leaves were placed in a 9 cm diameter Petri plate lined with Whatman No. 1 filter papers moistened with autoclaved distilled water. Mass inoculum of pathogen was prepared in 250 ml flasks containing 50 ml of ME broth. Flasks were inoculated with 2 mm discs of culture (2 discs) and then incubated at 25 ± 1°C for 5 days at 200 rpm. After the 5th day of growth, the fungal mycelium (1×10^5 spores) was sprayed over the different leaves samples. After 7-10 days, the treated leaves were compared with control leaves that were sprayed with water only.

Biocontrol of isolated fungi

Out of all the isolated cultures, *Phytophthora* species was observed as the major plant pathogen and was selected further for biocontrol studies. We have isolated three

strains of Trichoderma and therefore investigated for the biocontrol of *Phytophthora*. The *Phytophthora* inoculum was prepared in ME broth and after the 5th day of growth, 100 µl of spores suspension (1×10^5 spores) were spread onto the MEA plates and then Trichoderma species were inoculated with the help of inoculating loop over the spreaded suspension. After spot inoculation of Trichoderma, plates were incubated for 5 to 10 days at 25°C.

RESULTS AND DISCUSSION

Deodar, *C. deodara* (Roxb.), is a valuable timber tree which grows naturally at 1800 to 2600 m altitude in the North-west Himalaya. In this study, the site was selected 35 km from Rajgarh District Sirmour, Himachal Pradesh and the elevation ranged from 1900-2320 m altitude in the North-west Himalaya, where many deodar trees were infested. The disease has resulted in the decline and death of about 500 deodar trees. This huge loss was not only with looping caterpillars, but was also with fungi as well as mites. There might be some symbiotic relationship between the fungi, caterpillars and mites. The foliage in the infected trees turned yellowish, and the tops of the trees and tips of the branches dried up. Similar observation was reported by Alvidrez-Villarreal et al. (2012) in pecan (*Carya illinoensis*) wood and different pathogenic fungi associated to ambrosia borer were isolated.

Identification of different fungi

The isolated fungus includes 2 species of *Alternaria, Fusarium sp., Phytopthora sp., Aspergillus sp.* and 2 species of *Trichoderma*, though *Phytophthora* species was the most prevalent and isolated from all studied locations. Whereas the roots, wood as well as soil contained similar types of microflora namely, *Alternaria* predominant in all the samples followed by *Fusarium* and *Aspergillus*. On the contrary in the bark, the major microflora was *Trichoderma* species, which has close association with Lichens on the forest trees. Similarly, the results of Alvidrez-Villarreal et al. (2012) showed that the associated fungi to *Euplatypus segnis* which damaged the pecan wood were identified as: *Helminthosporium sp*., *Aspergillus sp*., *Penicillium sp*., *Phoma sp*., *Ascochyta sp*., *Phaecylomices sp*., *Umbeliopsis sp*., *Torula sp*., *Fusarium solani*, *Alternaria alternata*, *Fusarium oxysporum*, and *Lasiodiplodia theobromae*. Furthermore, Singh and Lakhanpal (2000) reported for the first time root rot disease of deodar *(C. deodara)* caused by pathogen *Phytophthora cinnamomi* from Himalayan region.

Defoliator *E. deodarae* (Lepidoptera, Geometridae) on *C. deodara* (deodar) in North-West Himalaya

In the pure deodar forest, outbreaks of this insect have occurred several times during the past few years. Recently, an outbreak was noticed in April, 2014 in different areas of Habban, Rajgarh region of Himachal Pradesh. The caterpillars were looper, light green initially and later turned pinkish brown and ultimately molted brown. The caterpillars fed by gnawing the needles from the tip to the base and scraped the basal portion of the needles; thereafter needles turned brown, dried up and fell down prematurely. The attack was so heavy that in about 810 ha of the area, complete defoliation of 80-100 year old stands, and observed the larval population was more than 2000-4000 per tree in May. In the later stages of attack, the branches of the trees were covered with the webs and veils of silk, and the plantation had a brown, scorched appearance. Afterwards as the raining season begins, all larvae pupate inside the soil near the tree trunk and deodar trees again regenerated after the raining season.

Females have semi-apterous abdomen with moderately robust long ovipositor, and the forewing are well developed whereas the hind wing is vestigial and not capable to fly. On the other hand, male moth has well developed wing and can fly. The males fly in the sky and to the top of the trees while wingless female crawls and climb on the tree trunk and lay eggs on the tender needles. The eggs are hatched in March-April and the caterpillar feed upon the foliage to the end of the raining season. The life cycle is annual. However, Singh (2007), and Singh and Verma (2008) reported similar observation during the *E. deodarae* outbreak on deodar in North-West Himalaya.

Pathogenicity test

The pathogenic leaves bioassay was performed on the Parthenium and 2 ornamental pine trees leaves. Out of all the cultures, *Phytophthora* showed maximum growth after 3rd day of fungal spores and ultimately after 7-10 days the leaves were totally defoliated. Similarly, *Alternaria* sp. also affected the leaves after the 5th day of fungal spray as compared to *Fusarium* and *Aspergillus* sp., which were affected after the 7th day but with lesser extent as compared to the *Phytophthora* sp.

Biocontrol of *Phytophthora*

Three species of Trichoderma were investigated for the biocontrol of *Phytophthora*. The Trichoderma species (a) inhibit the growth of *Phytophthora* almost 80%, whereas Species (b) and (c) were totally inhibitory to the *Phytophthora*.

Bisht et al. (2003) reported that inoculation with *T. viride* significantly controlled wilting of seedlings and improved plant growth in *C. deodara*. Microbial inoculations were found to influence the soil microflora, nutrient status of the rhizosphere soil and that of different parts of seedlings. Enrichment of carbon was recorded in the rhizosphere soil. Enhanced nitrogen, phosphorus and

potassium content of various plant parts indicated a positive influence also on nutrient uptake.

Singh et al. (2010) reported interactions between *P. cinnamomi* and antagonists (Trichoderma) involving coiling and parallel growth. Chambers and Scott (1995) also found that coiling, parallel growth and formation of apprisorium by *T. hamantum* and *T. pseudokoningii* inhibited growth of *P. cinnamomi*. There have been numerous reports of antagonisms of species of Trichoderma to pathogenic fungi including *P. cinnamomi* (Baker and Cook, 1974). On the contrary, Kelley (1977) concluded that neither *T. harzianmum* nor *T. polysporum* was significantly antagonistic to *P. cinnamomi*. However, Aryantha and Guest (2006) observed antibiosis as the main mode of action although mycoparasitism was indicated by parallel hyphal growth, hyphal coiling, appresorium formation and direct penetration with one isolate of *Trichoderma* against *P. cinnamomi*.

Conclusion

During the present study, epidemic defoliation of deodar forests by *E. deodarae* as well as different fungi have been isolated from the rhizosphere as well as non-rhizosphere soils, roots, bark and wood of *C. deodara* trees. *Phytophthora* spp. was the most prevalent and isolated from all studied locations. Furthermore, pathogenicity of all the isolated strains was tested and it was reported that *Phytophthora* are the most pathogenic strain though further identification is underway up to species level. In addition, *Phytophthora* will also be tested on Deodar Nursery Plants with further characterization underway. The long term studies and characterization of isolated fungal species will help in screening potentially pathogenic microbes and control the forest decline. The caterpillar life cycle as well as control measure with some entomopathogenic fungi will also be explored further.

ACKNOWLEDGMENTS

The authors acknowledge the respected Vice Chancellor, Dr. HS Dhaliwal and the Department of Biotechnology for providing the necessary research facility. They are also very grateful to the district forest officer (DFO), Y.P Garneria, Rajgarh District Sirmour, for allowing them to visit the site, as well as the forest rangers and guards for helping in sample collections.

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