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Studies on Fungal Associates of *Acacia catechu* (L.f.) Willd. – A Medicinal Plant

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ABSTRACT

Fungal associates of *Acacia catechu* growing in district Hamirpur of Himachal Pradesh were studied, identified and are being reported in the present research paper. The rhizosphere of *Acacia catechu* revealed the presence of twenty three species of fungi. Seven species of AMF (Arbuscular Mycorrhizal Fungi) belonging to three genera (*Acaulospora, Gigaspora* and *Glomus*) were isolated from roots adhering soil. Leaves and bark of this plant revealed the presence of three endophytic fungal species belonging to three genera namely, *Aspergillus niger, Cladosporium cladosporioides* and *Fusarium solani.* Further research of endophytes can be beneficial for the production of secondary metabolites and for bioprospecting.

***Key words**: Medicinal plant, AMF, Rhizosphere, Endophytes, Bioprospecting*

Introduction

The fungi occupy prime place in the biological world (Hawksworth *et al.,* 1995). They consist of large and heterogenous group of microorganisms permeating the surface layer of soil with their mycelium. Arbuscular mycorrhizal fungi (AMF) are ubiquitous, soil-borne, endophytic, obligate biotrophs that colonize the roots of most of the terrestrial plants in various soil types and environmental conditions to establish mutually beneficial relationships (Branco *et al.,* 2022; Shi *et al.,* 2023). Taxonomically, AMF belong to the phylum Glomeromycota (Giovannini *et al.,* 2020). The name AMF denotes the peculiar relationship between plant roots and mycorrhizal fungi and its key features, wherein "Arbuscule" stands for a special treelike fungal (hyphal) structure in the inner cortex of the root that is used for resource-exchange between "plant root" and "AMF," whereas "mycorrhiza" derived from two words "myco," meaning fungus, and "rhiza," meaning "root" (Shi *et al.,* 2023).

In plant-AMF symbiosis, host plant supply carbon (C) substances such as sugars and lipids to AMF (Jiang *et al.,* 2017). AMF provide mineral nutrients, particularly phosphorus (P) and nitrogen (N) to the host plant (Wipf *et al.,* 2019). This nutrient exchange strongly impacts plant and microbial ecosystems by influencing plant fitness, core soil processes and the C cycle (Diagne *et al.,* 2020; Giovannini *et al.,* 2020). AMF assist the roots of the host plant with their extensive hyphal network in absorbing water and nutrients, thus minimize the requirement for chemical fertilizers and irrigation (Kakouridis *et al.,* 2022). AMF are found in various kinds of habitats including severely disturbed ecosystems containing soils polluted with excessive salt, xenobiotics and heavy metals (Boorboori and Zhang, 2022; Branco *et al.,* 2022; Ahammed *et al.,* 2023). AMF help in plant development and stress tolerance such as drought

([1]Assistant Professor, [2]Assistant Professor) stress (Begum *et al.,* 2019), salinity (Cui *et al.,* 2022; Li *et al.,* 2022), nutrient deficiency (Shi *et al.,* 2021) or heavy metal stress (Dhalaria *et al.,* 2020; Alam *et al.,* 2019). AMF have potential applications in crop production, forestry management, bioremediation and ecological restoration as biofertilizers and bioprotectors (Phour *et al.,* 2020; Boorboori and Zhang, 2022; Zhu *et al.,* 2022). The effective management and assessment of ecosystem services offered by AMF is imperative for enhancing plant production both in terms of quality and quantity. Isolation of anticancer agent taxol from *Pestalotiopsis microspora*, an endophyte of Yew tree and phytohormone producing fungus from rice plant *Giberellafujikuroi* suggests the potential of endophytes as a source of useful metabolites (Stierle *et al.,* 1993). Present study was conducted to investigate the fungal associates (rhizosphere fungi, AMF spores and endophytic fungi) of an important medicinal plant *Acacia catechu.*

Materials and Methods

Sampling: Soil samples from the vicinity of roots of *Acacia catechu* were collected and examined for the presence of rhizosphere fungi and AMF spores. Leaves and bark of the tree were used for the isolation of endophytic fungi. Samples were collected during different seasons (winter, spring, summer and rainy).

Methods

Isolation of rhizosphere fungi: Dilution plate technique (Wakesmann, 1927 and Warcup, 1950) was followed. The medium used for culturing rhizosphere fungi was Potato Dextrose Agar (Rawling, 1933).

Assessment of VAM fungal colonization in roots: Root pieces were thoroughly washed with sterilized water and boiled at 90 ^{0}C for 1-2 hours in 10% KOH, acidified in 5N HCl, stained in

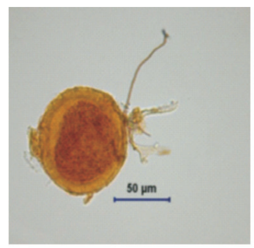
lactophenol trypan blue. The root segments were mounted on slides containing acetic acid: glycerol (1:1 v/v).

Isolation of VAM fungal spores: Wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was followed. Spores were identified according to size, shape and wall characteristics (Schenck and Perez, 1988).

Isolation of fungal endophytes: Hot water treatment and three step method was followed. In hot water treatment, leaves and bark were washed with hot water (60 ^{o}C) for 15 min and inoculated on Petriplates containing PDA medium (half strength). In three step method, samples were washed with sterilized distilled water and surface sterilized with 25% methyl alcohal (MeOH) for 5 min, followed by 50% MeOH for 3 min. and finally with 75% MeOH for 2 min. Finally these samples were washed in sterilized water for 5 min. and inoculated on Petriplates containing PDA medium (half strength).

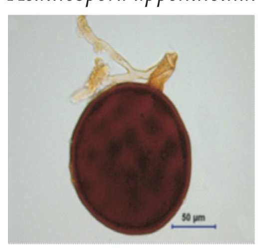
Results and Discussion

Twenty three species of rhizosphere fungi were isolated from the rhizosphere soils of *Acacia catechu*



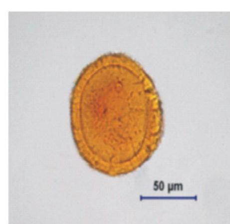
Acaulospora appendiculata

Gigasporacalospora

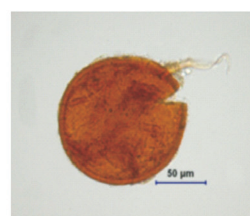


Glomus sp

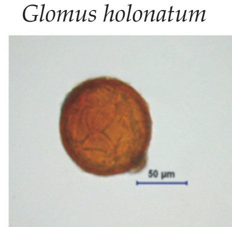
Glomus fulvum



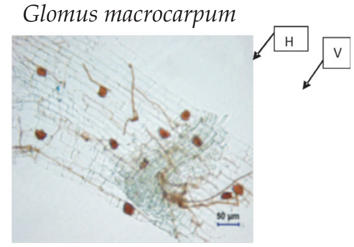
Glomus holonatum



Glomus macrocarpum



Glomus reticulatum



Hyphal coils (H) & vesicles (V) in root cortical cells of *Acacia catechu*

(Table 1). *Penicillium* was found to be most frequent genus followed by *Aspergillus*. Maximum diversity of rhizosphere fungi was recorded during rainy season whereas least in winter season. It was observed that maximum genera isolated during present study belong to subdivision Deuteromycotina. Behera and Mukerji (1984) reported that fungi imperfecti can tolerate wide range of environmental conditions as compared to fungi belonging to other subdivisions.

In the present study 7 species of AMF belonging to 3 genera (*Acaulospora*, *Gigaspora* and *Glomus*) were isolated (Plate 1). *Glomus* was found to be the most frequent genus. Sagar *et al.* (1993) and Kaur *et al.* (1997) while studying VAM associates of *Celtis australis*and*Grewia optiva*reported the genus *Glomus* to be more dominant in H.P. soils. Uniyal (2001) reported *Glomus macrocarpum, G. microcarpum, G. reticulatum, G. caladonium, Glomus* sp. and *Sclerocystis sinuosa* from rhizosphere of *Acacia catechu.*

Three species of endophytic fungi were also isolated from *Acacia catechu*,i.e. *Aspergillus niger, Cladosporium cladosporioides* and *Fusarium solani.* A comparison of the results of our study with the existing work of earlier workers revealed that majority of our results are in agreement with earlier investigations. However, some variations in the results can be attributed to adverse or favourable climatic conditions prevalent in this specific zone. The present study is helpful in obtaining preliminary information about fungal associates. These isolates can be utilized in the production of nursery seedlings and further investigation of endophytes can be beneficial for the synthesis of secondary metabolites and bioprospecting.

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Conflict of Interests

There is no conflict of interests regarding research, authorship and publication of this research article.

Table 1. Diversity and Seasonal Distribution of Fungi Isolated from therhizosphere Soil Samples of *Acacia catechu*

Sr. No.	Name of fungus isolated	Winter	Spring	Summer	Rainy
1.	*Alternaria alternata*	-	-	+	+
2.	*Aspergillus glaucus*	-	-	+	-
3.	*Aspergillus niger*	+	+	-	-
4.	*Aspergillus ustus*	-	-	-	+
5.	*Aspergillus wentii*	-	+	+	-
6.	*Aspergillus* sp.	+	+	+	+
7.	*Cunninghamella elegans*	-	+	+	+
8.	*Fusarium solani*	-	-	+	+
9.	*Gilmaniella humicola*	-	+	-	+
10.	*Gliocladium catenulatum*	-	+	-	-
11.	*Gliocladiumroseum*	-	+	-	+
12.	*Penicillium aurantiogriseum*	-	+	-	-
13.	*Penicillium citrinum*	+	-	+	+
14.	*Penicillium griseofulvum*	-	+	-	-
15.	*Penicillium restrictum*	-	-	-	+
16.	*Penicillium* sp. 1	+	-	+	-
17.	*Penicillium* sp. 2	-	+	+	+
18.	*Talaromyces* sp.	-	+	-	+
19.	*Talaromyces flavus*	-	+	-	+
20.	*Talaromyceshelicus*	+	-	-	+
21.	*Trematostroma* sp.	+	-	-	-
22.	*Trichoderma* sp.	+	-	-	+
23.	*Verticillium chlamydosporium*	-	-	+	-

+ = Present
– = Absent

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