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Fungal Associates of Medicinal Plant *Azadirachta indica* A. Juss

Sunil Pathak[1] and Babita Suman[2]

[1]Department of Botany, Govt. College, Barsar, H.P., India
[2]Department of Botany, NSCBM Govt. College, Hamirpur, H.P., India

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ABSTRACT

Fungal associates of medicinal plant *Azadirachta indica* A. Juss growing in district Una, Himachal Pradesh were investigated identified and are reported in the present paper. Twenty-two species of fungi were recorded from rhizosphere of *Azadirachta indica*. Seven species of Arbascular Mycorrhizal Fungi (AMF) belonging to three genera (*Acaulospora, Gigaspora* and *Glomus*) were isolated from root adhering soil of the plant. Leaves and bark of the plant revealed the presence of three endophytic fungal species belonging to three genera, i.e. *Fusarium, Gliocladium* and *Trichoderma*. Further study of endophytes can be useful for the production of secondary metabolites and bio prospecting.

Key words*: Fungal associates, AMF, Rhizosphere, Endophytic fungi, Secondary metabolites*

Introduction

The variety and galaxy of fungi occupy prime place in the biological world (Hawksworth *et al.,* 1995). Fungi form a 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). Systematically, AMF belong to the phylum Glomeromycota (Giovannini *et al.,* 2020). 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, especially phosphorus (P) and nitrogen (N) to the host plant (Wipf *et al.,* 2019). This nutrient exchange has strong impact on plant and microbial ecosystems as it influences plant fitness, core soil processes and the C cycle (Diagne *et al.,* 2020; Giovannini *et al.,* 2020). AMF aid the roots of the host plant with their extensive hyphal network in absorbing water and nutrients, thus decrease the need of 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 aid in plant development and stress tolerance such as drought (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 assess-

([1]Assistant Professor, [2]Assistant Professor)

ment of ecosystem services offered by AMF is imperative for enhancing plant production both in terms of quality and quantity.

Endophytic fungi isolated from *Azadirachta indica* have been reported to produce bioactive natural products (Li *et al*., 2007). Present studies was conducted to investigate the fungal associates (rhizosphere fungi, AMF and endophytic fungi) of important medicinal plant *Azadirachta indica*.

Materials and Methods

Sampling: Soil samples from the vicinity of roots of *Azadirachta indica* were collected and observed for the presence of rhizosphere fungi and VAM fungi. 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) was followed. Potato Dextrose Agar (Rawling, 1933) medium was used for culturing fungi.

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 fungi: Wet sieving and decanting technique (Gerdemann and Nicolson, 1963) was used for isolation of AM fungi. 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 ^{0}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% methanol (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-two species of rhizosphere fungi were isolated from the rhizosphere soil of *Azadirachta indica* during different seasons (Table 1). Fungal diversity was highest during rainy season while lowest during winter season.

Penicillium was found to be most dominant genus followed by *Aspergillus.* Rani *et al.* (2017) have islolated six species belonging to three genera (*Aspergillus, Fusarium* and *Penicillium*) from *Azadirachta indica*. It was observed that maximum genera isolated during present study belong to subdivision

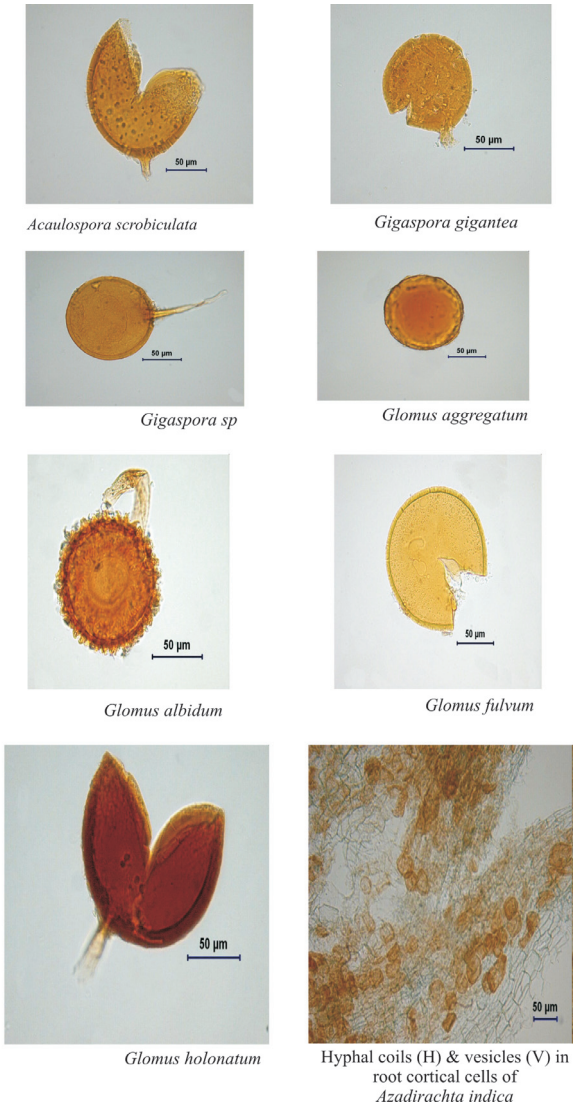

Plate 1. VAM Fungal Spores Isolated from the Rhizosphere Soil Samples

Deuteromycotina. According to Behera and Mukerji (1984) fungi imperfecti have high ability to tolerate wide range of environmental variations in comparison to other fungal species.

In the present study 7 species of VAM fungal spores belonging to 3 genera (*Acaulospora*, *Gigaspora* and *Glomus*) were isolated from *Azadirachta indica* (Plate 1).

Glomus was found to be the most frequent genus. Sagar *et al.* (1993) and Kaur *et al.* (1997) reported *Glomus* as most dominant genus in the soils of Himachal Pradesh while working on VAM associates of *Celtis australis* and *Grewia optiva.* Mohan *et al.* (1995) reported *Glomus aggregatum, Glomus fasciculatum, G. fulvus, G. macrocarpum, G. microcarpum, G. monosporum, G. occultum, Glomus* sp. and *Sclerocystis* sp. in rhizospehre of *Azadirachta indica* and found *Glomus* to be most frequent genus. Jite and Borde (2007) reported *Glomus* and *Sclerocystis* to be the most dominant genera in rhizosphere of *Azadirachta indica.*

In the present investigation 3 species of endophytic fungi were isolated from the leaves and bark of *Azadirachta indica i.e. Fusarium solani, Gliocladium catenulatum* and *Trichoderma viride*. However, contradictory to our results Rajagopal and Suryanarayanan (2000) isolated *Fusarium avenaceum* and two sterile endophytes from leaves of Neem. Mahesh *et al.* (2005) isolated 77 endophytic fungi belonging to 15 genera from inner bark of *Azadirachta indica*. Tejesvi *et al.* (2006) isolated species of *Chaetomium, Fusarium, Myrothecium, Pestalotiopsis, Trichoderma* and *Verticillium* in majority from inner bark segments of *Terminalia arjuna, Crataeva magna, Azadirachta indica, Holarrhena antidysenterica, Terminalia chebula* and *Butea monosperma.* Verma *et al.* (2011) have reported endophytic species of *Acromonium, Alternaria, Aspergillus, Cladosporium, Curvularia, Penicillium, Pestalotiopsis* and *Trichoderma* from roots and fruits of *Azadirachta indica*. 16 endophytic isolates were separated from *Azadirachta indica* by Taware and Rajurkar (2015). Taware *et al*. (2017) have reported *Alternaria* as dominant endophytic fungal species from *Azadirachta indica.*

In general, the results of our study are in agreement with the findings of earlier researchers. However, some variations in the results can be attributed to habitats or climatic conditions prevalent in this specific zone. The present study may be helpful in obtaining preliminary information about fungal associates. These isolates can be utilized in the produc-

Table 1. Diversity and Seasonal Distribution of Rhizosphere Fungi of *Azadirachta indica*

Sr. No.	Name of fungus isolated	Winter	Spring	Summer	Rainy
1.	*Aspergillus* sp. 1	+	+	+	+
2.	*Aspergillus* sp. 2	+	+	+	+
3.	*Aspergillus* sp. 3	-	+	-	-
4.	*Chaetomium* sp.	-	+	-	-
5.	*Coniothyrium* sp. 1	-	-	-	+
6.	*Coniothyrium* sp. 2	-	-	-	+
7.	*Fusarium solani*	-	+	-	-
8.	*Fusarium* sp.	-	+	-	-
9.	*Gilmaniella humicola*	-	+	-	+
10.	*Myrothecium roridum*	-	-	-	+
11.	*Penicillium aurantiogriseum*	-	+	+	-
12.	*Penicillium citrinum*	-	-	-	+
13.	*Penicillium restrictum*	-	-	-	+
14.	*Penicillium* with its perfect state *Talaromyces*	+	-	-	-
15.	*Penicillium* sp. 1	+	-	-	+
16.	*Penicillium* sp. 2	-	+	-	+
17.	*Penicillium* sp. 3	-	-	+	+
18.	*Phoma* sp.	-	-	+	-
19.	*Pythium* sp.	-	+	+	+
20.	*Trichoderma* sp.	+	-	+	+
21.	Nonsporulating sp. 1	+	+	+	+
22.	Nonsporulating sp. 2	+	+	+	+

+ = Present, – = Absent

tion of nursery seedlings and further investigation of endophytes can be useful for the large scale production of secondary metabolites and for bioprospecting.

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Conflict of Interests

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

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