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Endophytic fungal associations and VAM infection in the roots of *Murraya koenigii* (L.) Spreng. from the Hamirpur Distt of Himachal Pradesh

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Abstract
The impact of fungal endophytes on the leaves, bark, stem and root and vesicular arbuscular mycorrhizal fungal infection in the roots of *Murraya koenigii* was investigated. It has been found that both endophytic fungi and VAM fungi played an important role in the growth and development of the plant. Endophytic fungal species were isolated through three step method. Five species of endophytic fungi belonging to 4 genera (*Aspergillus, Alternaria, Fusarium and Trichoderma*) were isolated. The main species were *Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Aspergillus niger, Trichoderma viride* isolated from the leaves, bark, stem and roots of *Murraya koenigii*. *Aspergillus* and *Fusarium* belong to Ascomycotina and *Trichoderma and Alternaria alternata* belong to the Deuteromycotina. The VAM fungal infection in roots was also assessed by using Phillips and Hayman (1970) technique. Vesicles and fungal hyphae (mycelium) were observed in the roots of *Murraya koenigii.*

Keywords: Endophytic fungi, *Aspergillus, Alternaria, Fusarium, Trichoderma*, AM fungi

Introduction
Locally, *Murraya koenigii* plant is most commonly found in the lower parts of Himachal Pradesh. Its common name is Curry patta. It is a small tree with dark gray bark. The leaves are highly valued as seasoning in South Indian and Sri Lankan cooking, much like bay leaves and especially in curries with fish or coconut milk. The leaves of *Murraya koenigii* are also used as herb in Ayurvedic medicine. Their properties include much value as antidiabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, anti-hypercholesterolemic etc.

Over the past 20 years fungal surveys of various hosts have demonstrated that endophytic colonization of land plants by fungi is ubiquitous. Endophytes are known from plants growing in tropical, temperate, and boreal forests; from herbaceous plants from various habitats, including extreme arctic, alpine (Petrini 1987; Fisher *et al.* 1995) [14, 17], and xeric environments (Mushin and Booth1987; Mushin *et al.* 1989) [11, 12]; and from mesic temperate and tropical forests.

Microbes which live within the interior tissues of healthy plants without causing disease symptoms are called endophytes. Endophytic fungi colonize healthy tissues of plants belonging to different groups including algae, bryophytes, pteridophytes, gymnosperms and angiosperms (Johnson and Whitney, 1992) [3]. They survive with their hosts without producing any apparent symptoms or negative effects (Hirsch and Braun, 1992) [8]. Plant species having medicinal properties inhabit endophytes would provide various ample opportunities to discover new metabolites which has potential bioactivity. The inoculation of endophyte free leaves with endophytes isolated frequently from naturally asymptotic hosts significantly decreases both leaf necrosis and leaf mortality when *Theobroma cacao* seedling were challenged with a major pathogen of *Phytophthora* sps. (Arnold *et al.*, 2003) There are very few reports on the studies on endophytic diversity in *Murraya koenigii*. So, the present work has been done to explore its beneficial role in the growth and development of plant.

Arbuscular mycorrhizal fungi (AMF) are associated with almost all the plants in nature (Hayman, 1982) [7] Brundett *et al.*, 1996 studied the role of VAM that helped in increase the tolerance to adverse soil conditions and have compatibility with different hosts. They increase the plant productivity and are important for natural and managed ecosystems (Most of the major plant families are associated with mycorrhizal fungi involved in agricultural systems. However, 80% of plant species in surveyed lands are mycorrhizal (Wang and Qiu, 2006). AM colonization improved the drought tolerance of *Pistacia vera* seedlings by increasing the accumulation of osmotic adjustment compounds, nutritional and antioxidant enzyme activity. It appears that AM formation enhanced the drought tolerance of Pistachio plants, which increased host biomass and plant growth (Abbaspour *et al.*, 2012) [1]. The studies on association of AM fungi with sweet potato in soils of Andhra Pradesh revealed that AM fungal dependent and the edaphic factors such as soil pH, soil type, soil moisture content and soil nutrient availability influenced AM fungal distribution and colonization (Vogeti *et al.*, 2008) [20]. The influence of host on AM fungal diversity was studied and it has been found that indigenous population of AM was high at forest site followed by cultivated land, grassland and orchard. The different AMF genera were *Glomus, Gigaspora, Acaulospora, Entrophosphora* and *Scutellospora* (Chaturvedi *et al.*, 2009) [4]

Materials and Methods
Collection, Isolation and identification of Endophytic fungi
Materials used in the present study were roots, leaves, bark and soil samples from the rhizosphere of the medicinally important plant *Murraya koenigii* (L.) Spreng. Plants

growing in its natural habitat in different localities in Hamirpur. These samples were collected in polythene bags and were brought to the laboratory. The collected samples were first washed thoroughly in running tap water. Then the plant material (leaves bark and stem) was cut into small pieces (5mm approx.). About forty-eight pieces each were screened for the presence of fungal endophytes. These samples were washed with hot water (60^0C) for 15min. in a test tube then these samples were inoculated on petriplates containing PDA medium. PDA medium used was of half strength. Three pieces of each surface sterilized sample were then plated out on a petriplates containing PDA medium supplemented with Penicillin (150mgl^{-1}). These petriplates were incubated at 22±1^{0}C in incubator for one week. After fungal growth, sub culturing was done on PDA (Half strength) slants and the slants were preserved in refrigerator. These were identified under microscope by making a temporary mount.

Three Step Method

The samples were washed with sterilized distilled water. Then these were surface sterilized with 25% MeOH for 5min., followed by 50% MeOH for 3min, again followed by 75% MeOH for 2min. Finally these samples were washed in sterilized water for five minutes. Three pieces of each sample were then inoculated on petriplates containing PDA medium (half strength) amended with Penicillin (150 mg l^{-1}). Petriplates were incubated at 22±1^{0}C for few days. The fungal colony growing was then transferred on PDA slants (half strength). Temporary mounts of these were made in 0.1% Cotton Blue and Lactophenol and were then identified under microscope.

Assessment of VAM infection in Roots (Philips and Hayman, 1970)

The root pieces were thoroughly washed in tap water and boiled at 90^{0}C for 1-2 hours in 10% KOH depending upon the thickness of the roots. The root segments after washing in distilled water were acidified by immersing in 5N HCl for a few minutes. The root segments were then stained in 0.05% cotton blue in Lactophenol. The segments were cut into 1cm segments, which were mounted on slides containing acetic acid: glycerol (1:1v/v) and the edges were sealed with DPX mountant and then roots are examined under stereomicroscope for mycorrhizal association.

Results

Isolation and identification of different fungal endophytes of *Murraya koenigii*

It has been found that the endophytic fungi belonging to 4 genera (*Aspergillus, Alternaria, Fusarium, Trichoderma*) and 5 species (*Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Aspergillus niger, Trichoderma viride*) – were isolated from the leaves, bark and roots of *Murraya koenigii*. Two of these endophytic fungi belong to Ascomycotina (*Aspergillus* and *Fusarium*). Two other belong to the Deuteromycotina (*Trichoderma viride* and *Alternaria alternata*) (Table – 1, Plate-1).

Table 1: List of Fungal Endophytes of *Murraya koenigii*

Sr. No.	Endophytic fungus isolated
1.	*Alternaria alternate*
2.	*Aspergillus niger*
3.	*Fusarium moniliforme*
4.	*Fusarium oxysporum*
5.	*Trichoderma viride*

Photoplate I

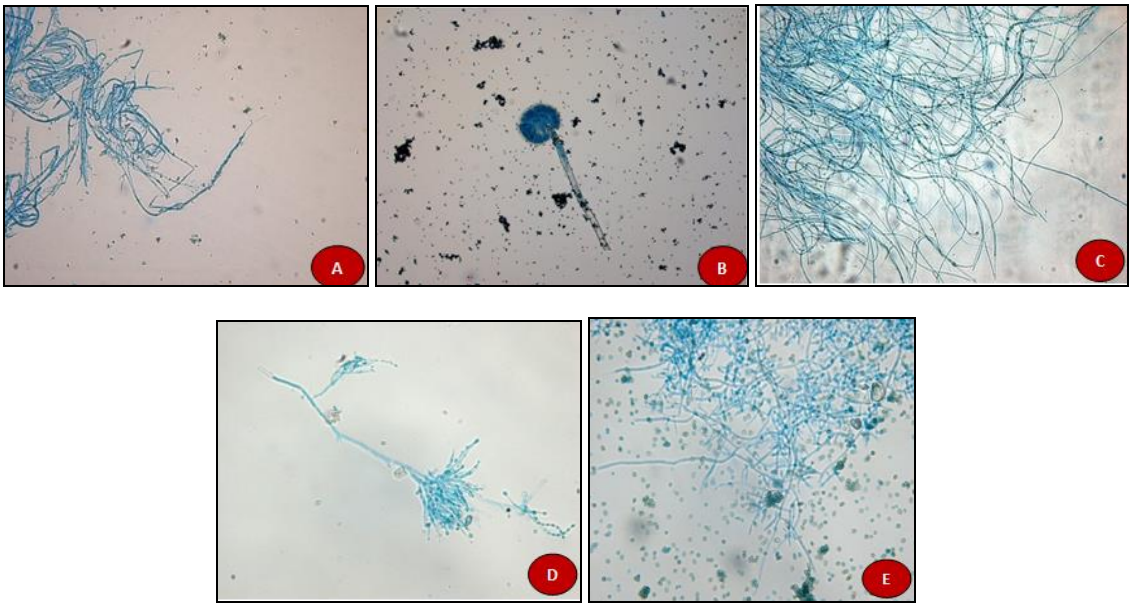

Fig 1: Photomicrographs of Endophytic Fungi showing (A) *Alternaria alternata* (B) *Aspergillus niger* (C*) Fusarium moniliforme* (D) *Fusarium oxysporum* (E) *Trichoderma viride*

VAM infection in the roots of *Murraya koenigii* (L.) Spreng.

VAM infection the roots of *Murraya koenigii* has shown that rainy season may considered as the best season for the propagation of plants by the application of AMF as bio-inoculants even for the plants of rare and threatened species.

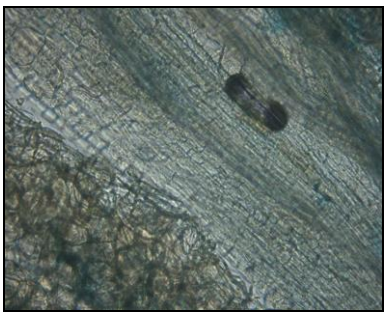

Fig 2: Photomicrographs of VAM Fungi showing VAM infection in the roots of *Murraya koenigii*

Discussion

Murraya koenigii is one of the important medicinal plants. The present results are based on the fungal isolations made from the rhizosphere soil samples and various plant parts like root, leaf and bark of *Murraya koenigii*. It has been found that five fungal species belonging to 4 genera were isolated as endophytes of *Murraya koenigii*. These fungal endophytes were *Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Aspergillus niger* and *Trichoderma viride* (Plate I, Table-1). Almost all vascular plant species harbor endophytic fungi or bacteria that are associated symbiotically or mutualistically without causing any noticeable diverse symptoms (Arnold *et al.,* 2003) [2].) *Fusarium solani* and *Cladosporium* sp. were isolated as fungal endophytes of *Azadirachta indica* and *Acacia catechu* (Suman, 2007) [18]. *Aspergillus niger* and *Fusarium* sp. were isolated as fungal endophytes of *Centella asiatica* and *Ocimum sanctum* (Kumari, 2007). Sagar and Thakur, 2007 isolated *Trichoderma* sp. was isolated as endophytic fungi from *Terminalia chebula*.

Sagar and Kaur (2009) [15] isolated *Aspergillus fumigatus, Fusarium oxysporum, Penicillium citrinum* and *Trichoderma pseudokoningii* from bark, leaves and roots of *Rhododendron arboreum*. Around six endophytic fungal species belonging to three genera of *Aloe vera* and *Mentha viridis* were isolated by Sagar and Thakur, 2009. These were *Macrophomina* sp, *Penicillium funicolosum* and *Trichoderma viride* which were isolated from *Aloe vera. Curvularia prasadii, Macrophomina* sp. and *Trichoderma fertile* were isolated from *Mentha viridis*.

Arbuscular mycorrhizal symbiosis played a crucial role inthe initial colonization of land by plants and in the evolution of the vascular plants. The AM fungi help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil. It is also apparent that rainy season may considered as the best season for the propagation of plants by the application of AMF as bio-inoculants even for the plants of rare and threatened species. An AM root is potentially capable of absorbing nutrients from soil *via* two pathways: directly into the root cells themselves and *via* the fungal symbiont (Smith *et al*., 2001). Mycorrhizal colonization of plants can offer considerable benefits in terms of growth and nutrient uptake (Gosling *et al.,* 2006) [6].

The findings of the present work are in the broad agreement with the reports of similar work done by the earlier workers. However, some variations in the results can be attributed to changes in habitats and climate factors of the specific region.

Conclusion

Fungal endophytes of *Murraya koenigii* were isolated from the leaves, bark and roots. Five species of fungi belonging to four genera were isolated as endophytes of *Murraya koenigii*. These were *Alternaria alternata, Fusarium oxysporum, Fusarium moniliforme, Aspergillus niger* and *Trichoderma viride*. Endophytic fungi colonize healthy tissues of plants such as leaves, twigs, bark etc. Although *Murraya koenigii* is one of the vastly studied plants but studies on interactions of endophytic fungi and VAM infection of this plant remain scarce. The present findings have shown vesicles and hyphae in the roots of *Murraya koenigii*.

References

1. Abbaspour H, Tolerance of mycorrhiza infected Pistachio (*Pistacia vera* L.) seedlings to drought stress under glass house conditions. J. Plant Physiol. 2012; 169:701-709.
2. Arnold AE, *et al*. Fungal endophytes limit pathogen damage in a tropical tree. *Acad. Sci.* USA. 2003; 100:15649-15654.
3. Brundrett M, *et al*. Working with mycorrhizas in forestry and agriculture. ACIAR Monograph, 32, 374+XP. ISBN 186320, 181**:**5.
4. Chaturvedi S, *et al*. Host-influences arbuscular mycorrhizal fungal diversity. J. Mycol. Pl. Pathol. 2009; 39:124-130.
5. Fisher PJ, *et al*. Fungal endophytes from the leaves and twigs of *Quercus ile* L. from England, Majoreab and Switzerland. *New Phytol.* 1994; 127:133-137.
6. Gosling P, *et al*. Arbuscular mycorrhizal fungi and organic farming. *Agric* Ecosyst Environ. 2006; 113:17-35.
7. Hayman DS. Practical aspects of VAM. In: Advances in agricultural microbiology. Subba Rao, N.S. (Ed). New Delhi Oxford, IBA, 1982, 325-373.
8. Hirsch GV, Braun U. Communities of parasitic microfungi. *In:* Handbook of vegetation science (Ed. W. Wintehroff). Dordrecht: Kluwer Academics Publishers, The Netherlands. 1982; 19:220-225.
9. Johnson JA, Whitney NJ. Endophytic fungi in algae. Can. J. bot. 1982; 70:1754-1755.
10. Kumari R. Studies on microbial associates of *Centella asiatica* and *Ocimum sanctum* M.Phil.Dissertation, Himachal Pradesh University, and Shimla, 2004.
11. Mushin TM, Booth T. Fungi associated with halophytes of an inland salt marsh, Manitoba, Canada. Can J Bot. 1987; 65:1137-1151.
12. Mushin TM, Booth T, *et al*. A fungal endophyte associated with a desert parasitic plant. Kavaka. 1989; 17:1-5
13. Phillips JM, *et al*. Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi from rapid

assessment of infection. Trans. Br. Myco. Soc. 1970; 55:158-161.
14. Petrini O. Endophytic fungi of alpine Ericaceae. *In:* Laursen GA, Ammirati JF, Redhead SA (Eds). The endophytes of *Loiseleuria procumbens*. *Arctic and alpine mycology* II. Plenium, New York, 1987.
15. Sagar A, Kaur R. Fungal associates of *Rhododendron arboreum* – A medicinal plant. Biozone Int. J. Life Sc. 2009; 1:75-84.
16. Sagar A, Thakur S. Studies on fungal associates of *Aloe vera* and *Mentha viridis*. Plant Archives. 2009; 9: 369-374.
17. Smith SE, Dickson S, Smith FA. Nutrient transfer in arbuscular mycorrhizas: how are plant and fungal processes integrated? Australian Journal of Plant Physiology. 2001; 28:683-694.
18. Suman B. Studies on microbial associates of *Azadirachta indica* and *Acacia catechu*. M.Phil. Dissertation, Himachal Pradesh University, Shimla, 2007
19. Thakur M, Sagar A. Fungal associates of *Terminalia Chebula*, *Terminalia* bellirica and Emblica officinalis. J. Pure and Appl. Microbio. 2007; 2:301-306.
20. Vogeti S, *et al*. Association of AM fungi with sweet potation soils of Andhra Pradesh. J. Mycol. Plant Pathol. 2009; 38(1):88-90
21. Wang B, Qiu YL. Phylogenetic distribution and evolution of mycorrhizas in landplants. *Mycorrhiza*. 2006; 16:299-363