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Fungal Associates of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*

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Fungal associates of three medicinal plants (*Harar, Bahera* and *Amla*) were isolated. Rhizosphere soil samples of *Terminalia chebula*, *Terminellia bellirica* and *Emblica officinalis* revealed the presence of thirteen, fifteen and thirteen species of fungi respectively. On screening these soil samples for the presence of VAM fungal spores, total seventeen species belonging to four genera (*Acaulospora*, *Entrophospora*, *Gigaspora* and *Glomus*) were isolated from these plants. Further seven genera of endophytic fungi were also isolated from the leaf, bark and root segments of *Terminalia chebula* (3 spp.), *Terminalia bellirica* (4 spp.) and *Emblica officinalis* (4 spp.).

Keywords: Rhizosphere, VA Mycorrhiza, Endophytes, Endomycorrhiza, Medicinal Plants.

Fungi are also known to colonize, multiply and survive in diverse habitats besides parasitizing plants as obligate parasites and biotrophs. Various kinds of mycorrhizal associations are known to be formed with vascular plants[1]. The rhizosphere (root soil interface) and VAM association of agricultural crops have been extensively worked out. So far, only few publications have reported the isolation of rhizosphere fungi, VAM spores and fungal endophytes (Fungi which live with in the interior tissues of healthy plants without causing disease symptoms) of ethnopharmaceutically important medicinal plants. Present communication records the fungal associates of three medicinal plants namely *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*. The fruits of these plants are the important constituents of "*Trifla*" a non habit forming rejuvenative[2].

Amla is used for the treatment of common cold, scurvy, cancer and heart diseases. It is believed that major constituent responsible for these activities is Vitamin C (Ascorbic acid) which shows antioxidant, anti-inflamatory and anti-mutagenic properties[3,4]. Fruits of *Terminalia chebula* are known for their laxative, astringent, alternative and stomachic properties and their cold infusion is used as a gargle in stomatitis, chronic ulcers, carious teeth, in cough, asthma and urinary diseases. The bark of *Terminalia bellirica* is useful in the treatment of anemia and leucoderma, fruits are bitter, pungent and are used as a medicine for dropsy, piles, diarrhoea etc[5].

In view of the medicinal importance of these three plants and reporting of taxol producing fungus *Taxomyces andraenae* from *Taxus brevifolia*[6] it was considered worthwhile to study the fungal associates of medicinal plants.

MATERIAL AND METHODS

Material used in the present study are roots, bark, leaves and soil samples from the rhizosphere of these plants.

For the isolation of the rhizosphere fungi, dilution plate method[7,8] was followed. Media used for culturing rhizosphere fungi were Czapek's

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Dox[9] and Potato Daxtrose Agar[10]. For isolating VAM spores from soil, Wet Sieving and Decanting Technique[11] was used. VAM infection in the roots was also assessed[12]. For the isolation of endophytes Hot Water Treatment and Three Step Method was followed. In Hot Water Treatment small pieces of leaves, bark and roots were washed with hot water (60°C) for 15 minutes in a test tube. Then these pieces were inoculated on petriplates containing PDA medium of half strength for endophytic fungal growth. In Three Step Method samples were washed with distilled water. Then these were surface sterilized with 25% methanol for 5 minutes, followed by 50% methanol for 3 minutes again followed by 75% methanol for 2 minutes. Finally these samples were washed in sterilized water for 5 minutes and then these samples were inoculated on petriplates containing PDA medium of half strength for growth of endophytic fungal colonies. Fungi were identified following (Trappe and Gilman)[13,14]. Identifications were authenticated by the scientists of A.R.I. Pune. Accession numbers have been taken for these fungi and the cultures have been deposited in the culture collection centre of the institute.

RESULTS AND DISCUSSION

13, 15 and 13 species of fungi belonging to 6, 7 and 7 genera were isolated from the rhizosphere soil samples of *Terminalia chebula* Table 1. *Terminalia bellirica* Table 2 and *Emblica officinalis* Table 3. Genera *Aspergillus* and *Penicillium* were found to be most frequent followed by *Trichoderma*. Maximum numbers of fungi were isolated during rainy season. VAM infection was observed in the root segments of *Harar, Bahera* and *Amla* (Fig. e, f and g). 17 species of VAM fungi belonging to 4 genera were isolated from the rhizosphere soil samples of these plants and *Glomus* was found to be most dominating genus represented by 9 species and it was followed by *Gigaspora* (3 spp.), *Acaulospora* (3 spp.) and *Entrophspora* (1 sp.) Table 4. Further, seven genera of endophytic fungi were isolated from the leaves, bark and root segments of *Terminalia chebula* (3 spp.), *Terminalia bellirica* (4 spp.) and *Emblica officinalis* (4spp.), Table 5.

A comparative study of different fungi isolated from the rhizosphere soil samples of these plants revealed that *Asergillus niger*, *Penicillium aurantiogresium* and *Trichoderma viride* were present in the rhizosphere soil samples of all the three plants. Non sporulating mycelia (one from each) were also isolated from the rhizosphere soil samples of these plants. Fungi *Emericella nidulans* and *Oedocephalum* sp. were isolated from the rhizosphere soil samples of *Terminalia chebula* only whereas *Absidia cylindrospora* and *Talaromyces flavus* were isolated from the rhizosphere soil samples of *Terminalia bellirica. Humicola grisea* and *Phoma* sp. were isolated from the rhizosphere soil samples of *Emblica officinalis* only. Lakhanpal and Kumar[15] isolated

Table 1. List of Rhizosphere Fungi Isolated from the Root Adhering Soil Samples of *Terminalia chebula*

S. No.	Name of the genera isolated	Name of the species
1.	*Aspergillus*	*A. niger, A. oryzae, A. versicolor, A. wentii*
2.	*Emericella*	*E. nidulans*
3.	*Fusarium*	*Fusarium* sp.
4.	*Oedocephalum*	*Oedocephalum* sp.
5.	*Penicillium sp.*	*P. aurantiogresium, P. citrinum, penicillium* sp. 1 and 2,
6.	*Trichoderma*	*T. viride* and *Trichoderm*a sp.

Table 2. List of Rhizosphere Fungi Isolated from the Root Adhering Soil Samples of *Terminalia bellirica*

S. No.	Name of the genera isolated	Name of the fungal species
1.	*Absidia*	*A. cylindrospora*
2	*Aspergillus*	*A. niger, A. oryzae, Aspergillus sp. 1* and *2,*
3.	*Cladosporium*	*C. cladosporioides*
4.	*Fusarium*	*F. equiseti*
5.	*Penicillium*	*P. aurantiogresium, P. chrichogenium, Penicillium* sp. 1, 2 and 3
6.	*Talaromyces*	*T. flavus*
7.	*Trichoderma*	*T. virde, Trichoderma* sp.
8.	Non sporulating mycelium	-

rhizosphere soil samples of *Terminalia chebula* only whereas *Gigaspora calospora*, *Gigaspora* sp., *Glomus fulvum* and *Glomus reticulatum* were isolated from the rhizosphere soil samples of *Terminalia bellirica* only. *Acaulospora scrobiculata*, *Acaulospora* sp., *Glomus fasciculatum, G. formosum, G. macrocarpum, G. segmentatum*, *Glomus* sp. 1, 2 and 3 were isolated from the rhizosphere soil samples of *Emblica officinalis* only. Uniyal and Uniyal[18] isolated *Glomus macrocarpum, G. reticulatum* and *Acaulospora scrobiculata* from the root adhering soil samples of *Dalbergia sissoo*. Tamuli and Boruah[18] isolated 2 genera *Glomus* and *Sclerocystis* from the rhizosphere soil samples of Agarwood tree. They found *Glomus* as most frequent VAM fungus in their investigation. Goje *et al.*[19] isolated *Acaulospora scrobiculata*, *Entrophospora infrequence* and *Glomus* spp. from the rhizosphere soil samples of *Phyllanthus niruri* and *P. emblica*.

Table 4. List of VAM Fungal Spores Isolated from the Root Adhering Soil Samples of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis*

S. No.	Name of VAM fungus isolated	*Terminalia chebula*	*Terminalia bellirica*	*Emblica officinalis*
1.	*Acaulospora appendiculata*	+	-	-
2.	*Acaulospora scrobiculata*	-	-	+
3.	*Acaulospora* sp.	-	-	+
4.	*Entrophospora*	+	-	-
5.	*Gigaspora calospora*	-	+	-
6.	*Gigaspora gigantea*	+	-	+
7.	*Gigaspora* sp.	-	+	-
8.	*Glomus fasciculatum*	-	-	+
9.	*Glomus formosum*	-	-	+
10.	*Glomus fulvum*	-	+	-
11.	*Glomus macrocarpum*	-	-	+
12.	*Glomus reticulatum*	-	+	-
13.	*Glomus rubiformis*	+	-	-
14.	*Glomus segmentatum*	-	-	+
15.	*Glomus* sp. 1	-	-	+
16.	*Glomus* sp. 2	-	-	+
17.	*Glomus* sp. 3	-	-	+

Table 5. List of Different Fungal Endophytes of *Terminalia chebula, Terminalia bellirica* and *Emblica officinalis*

S. No.	Name of fungal endophyte isolated	*Terminalia chebula*	*Terminalia bellirica*	*Emblica officinalis*
1.	*Aspergillus oryzae*	-	+	+
2.	*Baratalinia* sp.	-	-	+
3.	*Fusarium solani*	-	+	-
4.	*Pestalotiopsis* sp.	+	-	-
5.	*Penicillium* sp.	+	-	+
6.	*Rhizopus oryzae*	+	-	+
7.	*Trichoderma harzianum*	-	+	-
8.	*Trichoderma* sp.	-	+	-

* Agharkar Research Institute Fungal Culture Collection Number

A comparative analysis of endophytic fungal species isolated from root, leaf and bark of these plants revealed that fungus *Pestalotiopsis* was isolated as endophyte of *Terminalia chebula*. This genus was also isolated as endophyte from *Taxus wallichiana* a *Podocarpus nerrifolius* which have antitumour activities[20]. *Fusarium solani, Trichoderma harzianum* and *Trichoderma* sp. were isolated as endophytes of *Terminalia bellirica* only.

Tejesvi *et al.*[21] isolated *Pestalotiopsis* and *Fusarium* sp. as endophytes from the inner bark of ethnopharmaceutically important medicinal trees. *Baratalinia* was isolated as endophyte of *Emblica officinalis* only. *Aspergillus oryzae, Rhizopus orizae* and *Penicillium* sp. were also isolated in the present investigation.

Trichoderma spp. are reported to have growth promoting activities when cultivated with rice seedlings[22]. *Aspergillus* sp. and *Fusarium* sp. were isolated as endophytes from *Shorea robusta, Ptreocarpus marsupium,* and some members of family Combretaceae including *Terminalia chebula* and *T. bellirica*[23].The variations in the incidence of mycoflora under different parameters like rhizosphere, VAM fungi and endophytic studies may be due to difference in the root exudates[24], microbial competition, antagonism and succession.

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