Isolation & identification of soil fungi isolates from forest soil of Karnala Bird Sanctuary, Panvel

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Abstract

Soil in any type of terrestrial habitat, may be in agricultural fields, Forest land, grasslands, mangrove vegetation, deserts or sea beds is a living system & soil health in terms of soil quality& fertility results from physical properties, organic matter and the effective role of soil microorganisms which includes large species of bacteria and soil mycoflora. Soil fungi are very important as they are concerned with maintaining the soil parameters for healthy growth of forest trees. Fungi are the most common inhabitant of soil in the forests, decomposing leaves, branches, fruit pods by producing a wide variety of extracellular enzymes & can decompose dead organic matter into biomass & maintains nutrient balance.

Key words : Forest soil, Mycoflora, Soil fungi, Biological role, Seasonal variation.

Soil health, and the closely related terms of soil quality and fertility, is considered as one of the most important characteristics of soil ecosystems. The integrated approach to soil health assumes that soil is a living system and soil health results from the interaction between different processes and properties, with a strong effect on the activity of soil microbiota. All soils can be described using physical, chemical, and biological properties, but adaptation to environmental changes, driven by the processes of natural selection,

are unique to the latter one. This mini review focuses on fungal biodiversity and its role in the health of managed soils as well as on the current methods used in soil mycobiome identification and utilization next generation sequencing (NGS) approaches. In conclusion, the authors recommend a shift from cataloging fungal species in different soil ecosystems in forests toward a more global analysis based on functions and interactions between organisms. Forest soils are rich in fungi with utmost importance in providing humus a natural

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fertilizer to forest trees. Soil health, and the closely related terms of soil quality and fertility, is considered as one of the most important characteristics of soil ecosystems.

Decomposition by fungi is another important factor. Due to their ability to produce a wide variety of extracellular enzymes, they are able to break down all kinds of organic matter, decomposing soil components and thereby regulating the balance of carbon and nutrients Study area selected was Karnala Bird Sanctuary which is a reserved forest area with rich biodiversity and hence soil types. It is a reserved forest under Forest Department in the Panvel area of Raigad District. An exhaustive work has been done on the taxonomy of forest soil fungi in India. Saksena²⁰, Bakshi and Singh¹, Saksena and Sarbhoy¹⁹, Srivastava and Bhargava²², Gangawane and Deshpande⁴, Kamal and Bhargava^{6,7}, Manoharachary⁹, Madhusudan Rao and Manoharachary⁸, Manoharachary et al.,^{10,11}, Venugopal Rao et al.,²⁴ Reddy et al.¹⁷, Mohanty and Panda¹⁴ etc. have studied the soil fungi of different forests. Gauri Rane & Gandhe⁵ have investigated the seasonal distribution of soil fungi from the forest soils of Jalgaon. Very little information is available regarding biodiversity and taxo-ecology of soil fungi in the forest area of Raigad District. Hence the present investigation was undertaken for one year 2019-20.

Soil samples collected from various sites at Karnala Bird sanctuary were processed, cleaned, dried and sieved by using mechanical sieve to obtained fine particles. Physical analysis of the soil like color, texture, pH, salinity, maximum water holding capacity, Organic matter were studied. Soil samples were collected from the forest from four sites, at the depth of 8inch during all the three seasons - winter, summer and rainy season for the one year. These soil samples were collected in sterile bottles and were inoculated within 24 hours on nutrient media (Czapek-Dox Agar and Lactose Yeast Extract Agar) by Dilution Plate Method²⁵ and brought to pure culture for further studies.

Czapek- Dox Agar Composition :

- Agar, 15.0 g/L
- Di-potassium hydrogen phosphate, 1.0 g/L
- Iron (II) sulfate heptahydrate, 0.01 g/L
- Magnesium sulfate heptahydrate, 0.5 g/L
- Potassium chloride, 0.5 g/L
- Sodium nitrate, 3.0 g/L
- Sucrose, 30.0 g/L

Yeast Extract Agar :

- Agar, 15 g/L
- Peptic digest of animal tissue, 5 g/L
- Yeast extract, 3 g/L

Soil-dilution plate methods :

10 gms. of soil sample is taken in a 250 ml conical flask.100 ml of sterile water is added and the flask is vigorously shaken for a few minutes so that soil solution is obtained. This will represent 1:10 or 1/10. 10ml of the supernatant of this 1/10 solution is taken and 90 ml of sterile water is added and the resultant solution will be 1/100. In the same way, other dilutions like 1/1000, 1/10,000 and 1/100000 are made. One ml of the desired dilution is poured into a sterile petri-dish with 20 ml of melted and cooled agar medium. The petri-dish is rotated by hand to disperse the medium and the soil suspension.

The Petri-dishes are then incubated at 28°-30°C for a few days after which the growth of fungi takes place. Pure cultures of the fungi are then obtained by transferring them to agar slants.

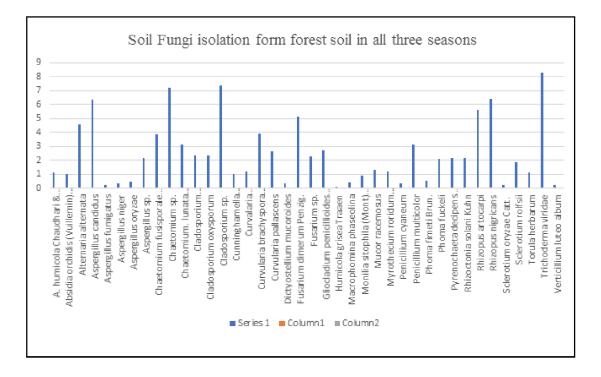
Fungal culture of pure form is obtained by inoculating a single spore or a piece of mycelium on the agar medium in tube slants or in Petri dishes. The inoculated agar plate is covered and the tube slant is plugged with nonabsorbent cotton wool.

They are then placed in an incubator which is usually maintained at a temperature of 30-37°C for a couple of days after which the agar plates and slants will show fungal growth in pure form.

The slants with fungal growth are then

send to Agarkar Research Institute, Pune for identification & photographs.

This method has been devised for determining the nature of the propagules (*i.e.*, spores or hyphae) that give rise to individual colonies of fungi on soil dilution plates. Soil dilution plates of clear, filtered Dox+yeast agar (pH $4 \cdot 2 - 4 \cdot 4$) are incubated for 18 hr. at 25° C, and are then searched for young fungal colonies, each of which is removed in a small agar block for direct microscopic examination. After the nature of the fungal propagule giving rise to the young colony has been determined, the agar block is transferred to fresh medium, to permit growth and identification of the fungus. Using this technique, the majority of the colonies developing on soil dilution plates were found to have arisen from spores.



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	29	Penicillium multicolor	-	-	+	3.16
31 Phoma fuckeli - + 2.11	30	Phoma fimeti Brun.	-			0.56
	31	Phoma fuckeli	-	-	+	2.11

32	Pyrenochaeta decipens Marchal	-	-	+	2.15
33	Rhizoctonia solani Kuhn	-	+	-	2.14
34	Rhizopus artocarpi	+	-	-	5.63
35	Rhizopus nigricans	+	-	+	6.38
36	Sclerotium oryzae Catt.	+	-	-	0.23
37	Sclerotium rolfsii	+	-	-	1.89
38	Torula herbarum	+	-	-	1.15
39	Trichoderma viridae	+	+	+	8.31
40	Verticillium luteo album	+	-	-	0.26
	Total				100%

The soil fungi isolated from selected sites in Karnala Bird Sanctuary, Panvel, showed significant diversity. Total 40 species of fungi belonging to 24 genera were recorded in one year study period from the forest soils of Karnala Bird sanctuary. The maximum contribution was made by *Trichoderma viridae* (8.31%) present in all the seasons, followed by *Cladosporium* sp. (7.36%), *Chaetomium sp.* (7.19%), *Rhizopus* nigrans (6.38%), *Aspergillus candidus* (6.32%), *Rhizopus* artocarpi (5.63%), *Fusarium dimerum* sp. (5.12%), *Alternaria alternata* (4.61%) and *Penicillium multicolor* (3.16%)

The dominant species in the present study was noted to be *Trichoderma viridae* present throughout the study period.

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