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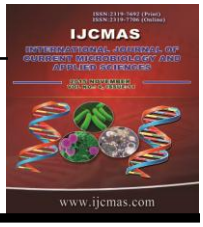

Original Research Article

Rhizospheric Fungal Diversity Associated with *Meyna spinosa* Roxb.: A Threatened and Ethno-Medicinally Important Plant of North-East India

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Keywords

Indo-Burma mega biodiversity hotspot, Rubiaceae, serial dilution plate method, Shanon-Weiner and Simpson diversity indices, species richness

A B S T R A C T

Meyna spinosa Roxb. belongs to Rubiaceae, is one of the threatened and ethno-medicinally important plant in North-East India, a part of Indo-Burma mega biodiversity hotspot. The plant is known for its pharmaceutical importance since it is used in the treatment of several human diseases such as hepatic disorders, gastro-intestinal problems, severe skin infections, diabetes etc. The plant is also important since it acts as refrigerant and abortifacient. In the present investigation, the rhizosphere of the test plant was screened for putative fungal associations using serial dilution plate method and 10^5 dilutions. Qualitative analysis revealed the predominance of nine fungal species belonging to eight different genera namely *Aspergillus*, *Cunninghamella*, *Geotrichum*, *Mortierella*, *Mucor*, *Penicillium*, *Pythium* and *Trichoderma* throughout the study locations. *Trichoderma* (50%) was recorded as the dominant fungal genera followed by *Mucor* (37.5%) and *Cunninghmella* (33.34%) respectively. The Shanon-Weiner and Simpson diversity indices for fungal isolates were high (1.979 and 0.847 respectively) at rhizospheric soil of *M. spinosa* collected from sampling point, JRT/NMT/MS-02. Significant variations in fungal species richness (3.0-16.6) throughout the study locations were also observed.

Introduction

Soil is a dynamic natural environment harboring a wide variety of life forms (Wilson, 1999). Rhizosphere is the active frontier of soil known for several biogeochemical reactions that have a tremendous influence on the natural biota associated with it (Morrissey *et al.*, 2004).

Hartmann et al (2008) reported the presence of unique microbial populations in the rhizosphere that are under the influence of chemicals released from plant roots. Rhizospheric microflora are directly associated with the growth inhibition of phytopathogens and synthesis of growth

promoting substances such as indole acetic acid (IAA) and giberellic acid (GA) (Bhattacharyya and Jha, 2012; Ahemad and Kibret, 2014). Stimulation of rhizosphere environment by plant root invasion is one of the significant contributions of rhizosphere microbiota towards development of quality soil for better crop improvement (Tkacz and Poole, 2015). Among the rhizosphere microbiota, a number of fungi are known for their significance in maintenance of nutrient cycling for sustainable ecosystem development (Read *et al.*, 2004). Fungi are also imperative in soil formation, maintenance of soil fertility and over all soil improvement (Hao-quin *et al.*, 2008).

India is one of the important mega biodiverse countries. North-east India is the bio geographical gateway of greater India that can be considered as one of the richest biodiversity hot-spot zone (Myers *et al.*, 2000; Bhattacharyya *et al.*, 2013) and is known for its potential genetic resources. However, studies pertaining to rhizosphere fungal diversities associated with medicinal plants are still scarce in India, the isolation and exploitation of which is important for better understanding on the mechanism of native mycoflora in influencing the microbial host relationship and physiology of the target plant species in a particular soil environment.

M. spinosa Roxb. belongs to the family Rubiaceae, is a thorny shrub known for its growth in hot and humid climate with slightly acidic to neutral pH ranging from 5.0-7.0. The plant is reported in India, Bangladesh, Nepal and in the plain lands of Java and Myanmar (Barbhuiya *et al.*, 2014). The plant has straight, sharp spines and whorled green leaves arranged in decussately opposite manner, whose flowering season generally starts in late spring and lasts until early summer. The fruits of the test plant are berry. *M. spinosa* is known for its active role in the treatment of several human diseases (Buragohain and Konwar, 2007), the fact which turned its significance in recent time. The present investigation has, therefore, been carried out with the objective to explore and characterize the native fungal diversities and its predominance pattern in the rhizosphere of *M. spinosa* Roxb., one of the threatened and ethno-medicinally important plant species of North-East India.

Materials and Methods

Collection of rhizospheric soil samples

Soil samples were collected using a randomized block design (RBD) from five different sampling points such as JRT/NMT/MS-01, JRT/NMT/MS-02, JRT/KKLM/MS-03, JRT/KKLM/MS-04 and JRT/SOT/MS-05 of Jorhat district (26°75′N-94°22′E), Assam, North-East India. Rhizospheric soil samples (three samples at each location) were collected by digging out a small amount of soil (approx. 500 g) from the depth of 15–30 cm using a sterilized hand auger. The samples were kept in pre-sterilized polythene bags and stored in a refrigerator at 4±1°C for further analyses.

Analysis of soil physico-chemical properties

The soil moisture was determined by drying 10 g fresh soil in a hot air oven at 150 °C until constant weight was obtained. Soil pH was recorded with an electric digital pH meter in 1:5 (w/v) soil-water suspensions at 22 °C. Soil temperature was determined at the time of sampling using a digital soil thermometer. For the estimation of Nitrogen (N), Phosphorus (P) and potassium (K), the soil samples were air-dried and sieved (0.2 mm). Total N was estimated by Indophenol blue method (Alien, 1974). The

molybdenum blue method (Jackson, 1967) was followed to determine the exchangeable soil P. Available K was extracted from the soil in an ammonium acetate solution (pH 7) and measured with a digital flame photometer (Systronic 121, India).

Isolation and enumeration of rhizospheric fungi

Serial dilution plate method (Johnson and Curl, 1972) and 10^5 dilutions were used to isolate the fungi from rhizospheric soil samples. Fungi were grown on potato dextrose agar (PDA) media using spread plate method (Pelczar *et al.,* 2005; John *et al.,* 2010). The plates were incubated at room temperature (25 ^oC) for 72 hours.

The number of colonies observed were counted and expressed in cfu/g fresh soil (Dubey and Meheshwari, 2005). Fungi were subcultured using sterilized inoculating loop and by streaking them on sterile petridishes containing PDA. For this, a small portion of the fungal hyphae was picked up with the aid of sterile inoculating needle and streaked on the surface of PDA plates and incubated at 25^oC for 72 hour. Sub culturing was continued until pure culture was obtained. The pure cultures, obtained were transferred into sterile PDA slants and incubated at 25 ±1^oC for 5 days. The agar slants were kept in a refrigerator at 4^oC for further identification.

Identification of fungal isolates

The fungal isolates were identified on the basis of their morphological and reproductive structures. Taxonomic monographs (Oilman, 1957; Subramanian, 1971; Ellis and Ellis, 1985; Domsch *et al.*, 2007) were used to identify the fungi. The fungi that did not produce spores were characterized as mycelia sterile. Percentage occurrence (%) of each fungal strain was determined in accordance with the following formula.

$$\text{Percentage occurrence (\%)} = \frac{\text{Number of a fungal species}}{\text{Number of colonies of fungal species isolated}} \times 100$$

Estimation of species diversity indices

Shannon-Wienner diversity index and Simpson's index of diversity were calculated using the following formulas.

$$\text{Shannon-Wienner diversity index, } H_s = -\sum_{i=1}^{S} (Pi)(\ln Pi)$$

Where, H_s = Symbol for the diversity in a sample of S species or kind.

S = The number of species in the sample.
Pi = Measures the relative abundance of i^{th} species or kinds = ni/N
N = The total number of individuals of all kinds.
ni = The number of individuals of i^{th} species.
ln = log to base 2.

Simpson's index of diversity = 1–D

Where, $D = \sum (n / N)^2$
n = The total number of organisms of a particular species.
N = The total number of organisms of all the species.

Results and Discussion

Analysis of rhizospheric fungal diversity

A good number of fungal isolates were recovered from the rhizosphere of *M. spinosa*. Figure 1 represents the photographs of some of the isolated fungal strains. Qualitative analysis revealed the predominance of eight fungal genera such as

Aspergillus, *Cunninghamella*, *Geotrichum*, *Mortierella*, *Mucor*, *Penicillium*, *Pythium* and *Trichoderma* in the plant rhizosphere (Figure 2). *Trichoderma* was recorded as dominant fungus in the present investigation. *Mucor* sp. (37.5%) and *Cunninghmella* sp. (33.34%), *Penicillium* (33.34%), *Pythium* (31.8%), *Aspergillus* (28.57%), *Geotrichum* (27.2%) etc. were the other fungal genera identified. A similar investigation on rhizosphere microbiota of wild medicinal legumes of barak valley was made by Singha and Sharma (2013) who, too, recorded altogether twenty different microbial isolates. Dominance of *Trichoderma* in the present investigation might be due to their greater rate of spore production and dispersal as well as their resistance in existing environmental condition. Rhizospheric fungal communities are one of the important biotic components in each and every ecosystem (Porras-Alfaro, 2011), since they are intimately associated with diverse biological processes like organic matter decomposition, recycling and transportation of nutrients, degradation of xenobiotic compounds etc. Altogether, nine species of rhizospheric soil fungi were isolated in the present investigation (Table 1). The study locations also showed marked variation in fungal species composition. *Trichorderma* showed maximum population density throughout the study locations. Evaluation of fungal diversity in the rhizospheric soil of *Ceropegia bulbosa* was made by Mulani and Turnkmana (2014). *Aspergillus* and *Mucor* were the dominant fungal genera recorded during their investigation. Dominant fungal species density in the present investigation was in agreement with Jha *et al.* (1992) and Bhattacharyya and Jha (2011) who, too, pointed out that for a given community only a few species are numerically predominant and may strongly affect the environmental conditions for the others.

The Shanon-Weiner and Simpson diversity indices were also showed distinct variations throughout the study locations (Table 2). Shanon-Weiner and Simpson diversity indices were high at sampling location 02 (JRT/NMT/MS-02), thus, indicating high species diversity in that particular location. Species richness was also high at that location. Diversity analysis for soil fungi isolated from different land use systems of North Brahmaputra Valley, Assam was also made by Bhattacharya (2012), thus, indicating a significant diversity pattern for isolated fungal strains. More diverse fungal isolates and high species richness at sampling point 02 might be attributed to the presence of more organic nutrients in soil (Hartmann *et al.*, 2015).

Physico-chemical properties of soil

The physico-chemical properties of the collected soil samples are represented in table 3–4. The soil pH ranged from 4.0 to 4.5 respectively, thus, indicating the acidic nature of soil (Table 3). Carbon qualities of the rhizospheric soil ranged from 1.21–3.95 %. It was observed that the rhizospheric soil sample of *M. spinosa* contains more N (380.93Kg/ha) followed by K (366.64 Kg/ha) and P (30.4 Kg/ha) respectively (Table 4). Analysis of soil physico-chemical parameters for estimating the diversity of AMF associated along with some medicinal plants are also made by Rajkumar *et al.* (2012), who, observed slight elevation in soil P level. Variations in soil physico-chemical properties throughout the study locations might be due to incomplete oxidation or decomposition of organic matter in rhizospheric soil. In addition, topography might influence the quantity and diversity of fungal population numbers in rhizospheric soil (Tsai *et al.*, 2007).

Table.1 Population density of the fungal species at different sampling locations

Fungal compositions	Sampling locations				
	JRT/NMT/MS-01	JRT/NMT/MS-02	JRT/KKlM/MS-03	JRT/KKLM/MS-04	JRT/SOT/MS-05
Oomycota- 01 genus and 01 species					
Pythium irregular	++	+	+	-	+
Zygomcota- 03 genera and 03 species					
Cunninghamella sp.	-	+	-	+	+
Motierella horticola	-	+	-	-	-
Mucor racemosus	-	++	+	-	++
Ascomycota-05 genera and 05 species					
Aspergillus ochraceus	++	++	+	+	++
Geotrichum candidum	+	+	+	-	-
Oidiodendron Sp.	+	-	-	-	-
Penicillium crateriforme	+	+	-	-	-
Trichoderma harzianum	+++	++	+++	+++	++

Where, - = Absent
++ = High fungal population density (07-12 isolates)
+ = Low fungal population density (01- 6 isolates)
+++ = Very high fungal population density (13-18 isolates)

Table.2 Species richness and diversity of rhizospheric fungi throughout the study locations

Sampling locations	Total isolates	Species Richness	Diversity Indices	
			Shannon	Simpson
JRT/NMT/MS-01	08	11.5	1.733	0.813
JRT/NMT/MS-02	12	16.6	1.979	0.847
JRT/KKlM/MS-03	06	9.3	1.561	0.778
JRT/KKLM/MS-04	02	3.0	0.693	0.5
JRT/SOT/MS-05	07	9.6	1.55	0.776

Table.3 Physical parameters of the rhizospheric soil samples

Study locations	Soil pH	Soil temperature (ºC)	Electrical conductivity(EC) (dS/m) SO
JRT/NMT/MS-01	4.0± 0.5	20±0.9	0.08±0.1
JRT/NMT/MS-02	4.3±1.5	20±1.1	0.09±0.6
JRT/KKlM/MS-03	5.2±0.9	20±1.3	0.08±0.7
JRT/KKLM/MS-04	4.48±0.7	28±1.4	0.08±0.4
JRT/SOT/MS-05	4.95±0.9	29±0.7	0.22±0.3

Data are the mean of three replicates, ± S. D.

Figures.1 (A-F): Photographs of isolated fungal strains. A: *Cunninghamell*a sp., B: *Aspergillus flavus*, C: *A. ochraceus*, D: *Trichoderma viride*, E: *Basidiobolus* sp., F: *Mortierella* sp.

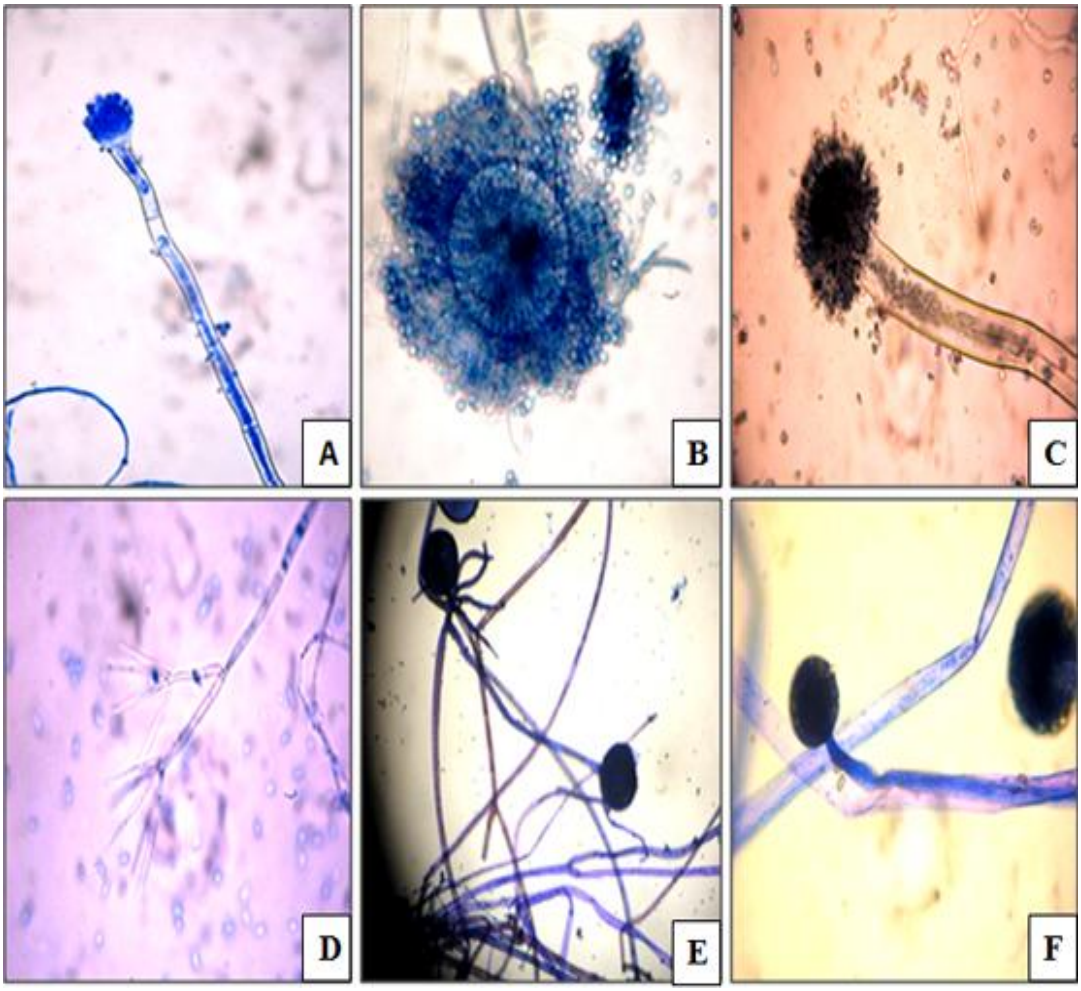
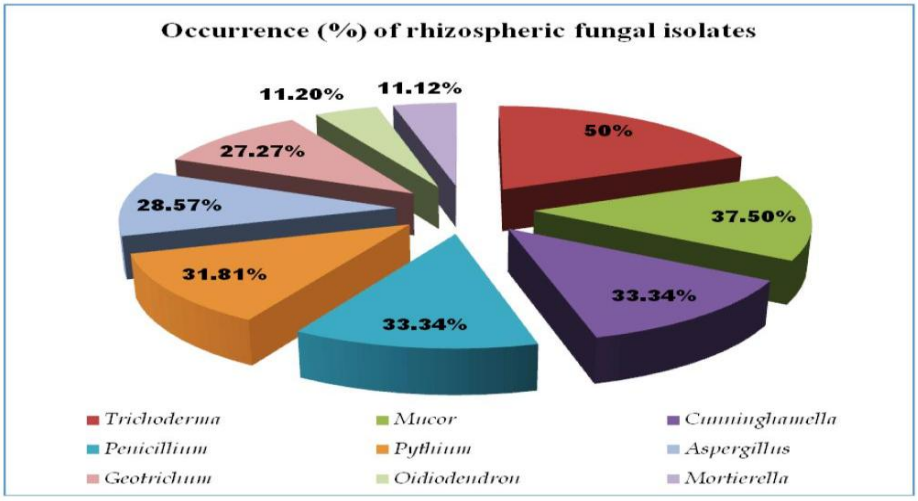


Table.4 Chemical analysis of the rhizospheric soil samples

Study site	C_{org} (%)	Available N (Kg/Ha)	Available P (Kg/Ha)	Available K (Kg/Ha)
JRT/NMT/MS-01	0.84±1.3	445±1.3	29.5±2.1	366.64±0.9
JRT/NMT/MS-02	0.82±1.9	442±0.2	30±1.6	408.84±0.7
JRT/KKlM/MS-03	1.14±0.5	457±0.7	36±1.1	378±0.5
JRT/KKLM/MS-04	0.87±0.9	490±1.1	28±0.9	384.4±0.3
JRT/SOT/MS-05	0.81±1.4	448±0.9	28.7±1.2	366.76±1.1

Data are the mean of three replicates, ± S. D

Figure.2 Percentage occurrence (%) of fungal isolates in the rhizosphere of *M. spinosa*



In conclusion, the present investigation thus indicated the significance of rhizosphere of *M. spinosa* as one of the potent reservoir of mycofloral diversity, the isolation and identification of which seemed to be important for future studies related with phsiolological limits of microbial life, microbial mechanism of action and potentially analogous environment for sustainable human welfare.

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