

Locational variation in rhizospheric mycobiota associated with *Elaeagnus latifolia*, an endemic plant species of N.E. India

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Abstract

Mycobiota are a group of all fungi present in a particular geographic region or habitat type and the most richest type of mycoflora is found in the rhizosphere. This mycobiota along with the rhizospheric environment plays a significant role in growth and development of plants. As a result, the current study was conducted to evaluate the rhizospheric mycobiota linked to *Elaeagnus latifolia* L. on a quantitative and qualitative level. In this study, the rhizospheric mycobiota were enumerated and it was found that a total of 46 species of fungi belonging to 19 families and 4 orders were associated with the target plant species. Variations were observed in the density, abundance, natural occurrence and diversity indices of the studied species in correlation to the location, habitat and elevation wise parameters. The study revealed that a large number of beneficial mycobiota are associated with the species which can be further explored and exploited for potential usage as bioagents, biofertilizers and biopesticides for a sustainable forestry and towards a green environment.

Keywords: bastard oleaster; microbial dynamics; microbiology; mycobiota; rhizosphere

Introduction

The rhizosphere is the zone of soil surrounding a plant root (Subba Rao, 1993). It is an area of intense biological and chemical activity influenced by compounds exuded by the plant roots and microorganisms such as bacteria, actinomycetes, fungi, slime moulds, algae, viruses etc. Rhizospheric soil is one of the richest sources of microbial community and these soils possess very huge diversity of both beneficial and pathogenic microbes (Katoch *et al.*, 2020). According to Walker *et al.* (2011) rhizosphere includes the region of soil bound by plant roots, often extending a few millimetres (mm) from the root surface. As plant roots grow, they release water soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. The microorganisms are known to provide nutrients for the growing plants. Rhizospheric microorganisms are also known to produce vitamins, antibiotics, plant growth regulators and signal molecules that can influence plant growth significantly (Bhattacharyya and Jha, 2012). Microbial secretions around the rhizosphere are also helpful in combining the soil particles into stable aggregates to retain soil moisture. Rhizospheric microorganisms are considered pivotal for plant health and growth due to their involvement in such key

processes as the formation of the root architecture, formation of soil characteristics; decomposition of organic matter; decomposition and removal of toxins, defense against plant pathogenic microorganisms and pests; and cycling of carbon, nitrogen, phosphorus and sulphur (Qiao, 2019). It has been shown that fungal interactions and community diversity can promote plant diversity and productivity but can also have potential negative effects through actions of plant pathogenic fungi. Thus, the functionality of the mycobiota in the rhizosphere affects both individual plants and plant community function. Furthermore, root-associated fungi can form an important link in the flow of energy and nutrients from plants to predatory soil organisms. Together, these plant-microbe consumer effects create complex multi-trophic interactions in the rhizosphere (Hannula *et al.*, 2020).

Microbial interactions in rhizosphere may be pathogenic that may invade or kill plant roots or beneficial that directly or indirectly promote the growth of the plants. They may be saprophytic that live on dead residues of plant bodies or neutral causing no visible effect to the plant. Thus, rhizosphere has appeared as a versatile and dynamic ecological environment of intense plant-microbe interactions (Mayak *et al.*, 2004). Microbial communities, particularly bacteria and fungi constitute an essential component of biological characteristics in soil ecosystems (Swier *et al.*, 2011). However, fungi play an important role in terrestrial ecosystems or soil ecosystem as major decomposers of plant residues, releasing nutrients that sustain and stimulate plant growth in the process. In the study of soil fungi, particular attention is given to the rhizosphere (Couteaudier and Alabouvette, 1990), where they mediate many ecological processes, and are influential for plant growth and soil health (Zachow *et al.*, 2009). There is a close link between plant species and microbial community structure in the rhizosphere, and the hypothesis is that bacteria and fungi have also developed a unique diversity pattern over time, which may give insights into microbial evolution (Zachow *et al.*, 2009). Thus, the functional diversity of different fungi present in the soil should be studied so that the beneficial effects of this rhizospheric microflora are fully exploited and utilized.

In this investigation, an attempt is made to study the soil ecology of *Elaeagnus latifolia* L. and reveal the micro-fungal diversity associated with it. *Elaeagnus latifolia* L. belongs to family *Elaeagnaceae*, locally known as *Soh-shang* in Khasi hills of Meghalaya and *Mirika Tenga* in Assam, is quite uncommon in Sibsagar (Dikho valley of Assam), Naga hills (Nagaland), Khasi and Jaintia hills of Meghalaya up to an elevation of 1500 above mean sea level (msl) of North- East India. In recent years, *Elaeagnus latifolia* have declined rapidly in number and population of plants from many locations. The risk of the disappearance may be as an endemic plant species with restricted geographic distributions and rare plant species endangered by habitat destruction (Handique and Parkash, 2014). They studied the rhizospheric root samples of the plant species and found that it had all the three types of root infection/colonization namely hyphal infection (90%), arbuscular infection (70%) and vesicular infection (25%). The qualitative analysis revealed that the predominant endomycorrhizae in the rhizosphere belongs to three genera viz. *Glomus*, *Acaulospora* and *Gigaspora*. Altogether, 13 species of AM fungi were observed viz. *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. macrocarpum* Tulasne & Tulasne, *G. multicaule* Gerd. & Bakshi, *G. constrictum* Trappe, *Acaulospora laevis* Gerdemann & Trappe, *A. lacunosa* Morton, *A. appendicula* Spain, Sieverding & Schenck, *A. mellea* Spain & Schenck, *A. bireticulata* Rothwell & Trappe, *Acaulospora* sp., *Glomus geosporum* Walker, *Gigaspora* species. The diversity of arbuscular mycorrhizae was retained by *Acaulospora* spp. (46.6%), followed by *Glomus* spp. (40%) whereas the occurrence frequency of *Gigaspora* spp. (13.3%) was found to be the least among all the three genera. Moreover, the plant species is an actinorhizal plant species which is known to harbour the actinomycetes bacteria species *Frankia* sp. and a very few species in north east India are known to have this symbiotic associations. Further, they reported that the plant species is associated in a number of mutualistic relationship with actinorhizal, endomycorrhizal and endophytic microbiota. Such parameters establish it as a model host for ecological succession. Due to its ability in fixing atmospheric nitrogen and phosphorus intake capacity *Elaeagnus latifolia* can also be used as a model system for agroforestry programmes such as in tea gardens etc. It can be speculated that such associations can play a vital role in bioactive compounds production too as it is an important medicinal plant species. Thus, *Elaeagnus latifolia* L. was chosen as the targeted plant species.

Materials and Methods

Study Area

The entire study was conducted at three geographical locations of Northeast India such as Jorhat (one of the richest biodiverse zones in Assam with a geographical area of 2859.3 sq. Km lies between 26.46 °N latitude and 96.16 °E longitude and 116 m above mean sea level), Sivasagar (26.45 °N latitude and 95.25 °E longitudes and 86.6 m above mean sea level), and Meghalaya (25.65 °N latitude and 91.88 °E longitude and 1525 m above mean sea level) (Figure 1). Survey was made at selected areas for the collection of rhizospheric soil samples. In the present investigation, a culture-based approach was primarily used to isolate and characterize the rhizospheric mycobiota of selected study points.

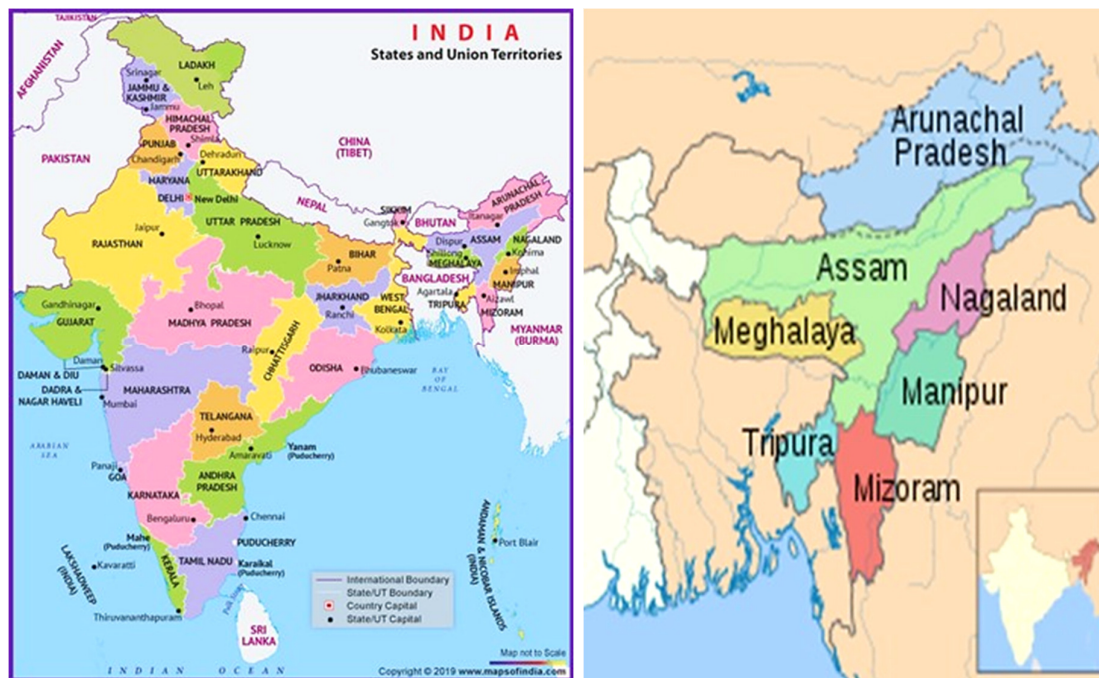


Figure 1. Study site: Map of India (left); Meghalaya and Assam locations (right)

Collection of Soil Samples

The study was conducted in five selected sites under Assam and Meghalaya states, India and five numbers of samples of the target plant species along with their collection/accession number have been collected. Wild areas where this plant species is naturally growing in the district Jorhat and adjoining areas were explored for the collection of the target plant species and the rhizospheric soil samples. The accession/collection numbers of the collected samples are as follows JRT/CHN/FS-01; JRT/BSP/FS-02; SHG/NEHU/FS-03; JRT/RFRI/FS-04; and SBS/SOT/FS-05. The Collection number and accession numbers were given in accordance to the site of collections from which the soil samples and plant samples were collected from. Moreover, the habitat wise and elevation wise occurrence of the rhizospheric mycobiota were also studied.

Rhizospheric soil samples (at least three samples at each location) were taken by digging out a small amount of soil (500 g) adjacent to plant roots up to the depth of 15-30 cm. The randomly collected soil samples were mixed properly and passed through a 2.0 mm sieve to remove the debris. 1.0 g of soil was then suspended in 10 ml of Sterile distilled water (SDW) and incubated in an orbital shaking incubator at 28 °C with periodic shaking at 200 rpm (rotation per minute) for 30 minutes. 10-fold series dilutions were prepared and the soil

suspension of required dilution was then inoculated into culture media and incubated at optimum temperature for maximum growth of the micro fungi. The colony forming units (cfu) were counted after proper incubation period was over.

Analysis of Soil Physico-Chemical Properties

Soil pH

To determine the soil pH, 5 g of soil sample is taken in a test tube with equal amount of BaSO₄ (Barium sulphate). 10 ml of distilled water is then added to the mixture and shaken well. The whole mixture is kept for 10 minutes. The supernatant is collected in a test tube and 1-2 drops of universal indicator is added to it. By matching the colour of the supernatant with pH chart, the pH of the sample was recorded.

Soil Moisture (%)

Soil moisture (%) was determined by drying 10g of fresh soil in a hot air oven at 150 °C until constant weight is obtained. The percentage of moisture content was calculated by using the following formula:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where,

W₁ = Weight of the soil before drying,

W₂ = Weight of the soil after drying.

Estimation of Soil Organic Carbon (C_{org} %)

Walkley and Black (1934) rapid titration method was followed to determine the organic carbon present in soil. For this, one gram of air dried, sieved soil was taken along with 10 ml of 1N K₂Cr₂O₇ and 20 ml of conc. (concentrated) H₂SO₄ in a 500 ml conical flask. The flask was left for 30 minutes. 10 ml of 85% phosphoric acid was then added to it and titrated with 1N FeSO₄ solution using diphenylamine as an indicator. Same procedure was followed for the blank solution. Three replicates were maintained in each case. Soil organic carbon (C_{org} %) was estimated using the following formula:

$$\text{Corg \%} = \frac{B - S \times 0.003}{W} \times 100$$

Where,

B = Volume of FeSO₄ used for the blank titration,

S = Volume of K₂Cr₂O₇ used for the sample titration,

W = Weight of the soil sample.

Determination of Total Nitrogen (N_{tot} %)

To determine the total Nitrogen, 0.1 g ground soil was taken in a micro-Kjeldahl flask and 2 g of K₂SO₄: HgO (20:1) mixture was added to it. 3 ml of conc. H₂SO₄ was slowly added in the flask and rotated carefully. Thereafter, the flask was gently heated on a digestion rack. Heating was continued even after the digest became colourless. After digestion, the digested material in the flask was diluted with sterile distilled water (SDW) and filtered through Whatman No. 1 filter paper. Thus, due to digestion, all the organic nitrogen present in the sample got converted into ammonia that was measured by Indophenol blue method as suggested by Allen et al. (1974). While, blank digestion was prepared only with the reaction mixture. Total nitrogen (N_{tot}%) was calculated using the following formula:

$$\text{N tot \%} = \frac{\text{Organic nitrogen (mg)} \times \text{Solution volume (ml)}}{10 \times \text{Aliquot (ml)} \times \text{Sample weight (g)}} \times 100$$

Determination of Exchangeable Phosphorus (ppm/g dry soil)

Molybdenum blue method (Jackson, 1973) was followed for the determination of exchangeable phosphorus. For this, 1.0 g of air dried, sieved soil was taken in a 500 ml conical flask, where 200 ml of extraction solution was added and stirred for 30 minutes. Thereafter, the mixture was filtered through Whatman No. 42 filter paper. 30 ml aliquot was then pipetted in 50 ml volumetric flask containing 2-3 drops of dinitrophenyl indicator. Further, 2N H₂SO₄ was added to the solution, till it became colourless. 2 ml of sulphomolybdic acid was added to this solution. The flask was shaken well and 0.5 ml of chlorostannous acid was added to it and the final volume was maintained to 50 ml by adding SDW. Absorbance of the solution was read in a spectrophotometer at 660 nm. Exchangeable phosphorus (P) was calculated using the following formula:

$$\text{Exchangeable P (ppm/g soil)} = \frac{\text{P (ppm)} \times \text{extraction solution (ml)}}{\text{Weight of the sample (g)} \times 10 \times \text{aliquot (ml)}}$$

Determination of Available Potassium (ppm/g dry soil)

Potassium was extracted using ammonia acetate solution (pH 7) that was prepared by mixing 575 ml of glacial acetic acid with 600 ml of ammonia solution. The mixture was diluted up to 10 litres by adding SDW. For the determination of available potassium, 1.0 g of sieved soil was added to 125 ml of this extraction solution and stirred constantly for about 1 hour. The extraction mixture was then filtered through Whatman No. 44 filter paper. Available potassium was determined according to the following formula:

$$\text{Available K (ppm/g soil)} = \frac{\text{K (ppm)} \times \text{solution volume (ml)}}{10^4 \text{ dilutions} \times \text{sample weight (g)}}$$

Growth Media, Isolation and Culture Conditions for Rhizospheric Fungi

Potato dextrose agar (PDA) was used for the isolation of soil fungi using dilution plate technique (Johnson and Curl 1972) and 10⁵ dilutions. The media were supplemented with 50 µg/ml of streptomycin sulphate to prevent bacterial growth. Fungi were grown at 25±1 °C for 5 days. Three replicates were maintained in each case. Pure colonies were transferred to PDA slants overlaid with mineral oil and stored at 4 °C for further identification.

Identification of the Isolated Rhizospheric Mycoflora

Fungal isolates from rhizosphere were identified macroscopically based on their cultural, morphological, colony morphologies, including texture, color, radial and concentric lines. The microscopic identification was performed by viewing the hypha, sporangium, conidiophore and conidia by consulting various taxonomic monographs (Gilman 1957; Subramanian 1971; Watanabe 1993 and 2002; Domsch et al. 2007). The fungi that did not produce spores were characterized as mycelia sterile and those showing no diagnostic morphological characters were included under unidentified strains.

Quantitative Analysis

Quantitative analysis of parameters like density, frequency and abundance of the microbial populations were determined in accordance with Curtis and McIntosh (1950).

(a) Density

Density is the expression of numerical strength of a species where the total number of individuals of each species in all compartments is divided by the total number of compartments studied. Density is calculated by the following equation:

$$\text{Density} = \frac{\text{Total number of individuals of a species in all compartments}}{\text{Total number of compartments studied}}$$

(b) Frequency (%)

Frequency refers to the degree of dispersion of individual species in an area and usually expressed in terms of percentage occurrence. It is generally estimated by sampling the survey area into several places randomly and the name of the species that occurred in each sampling unit is, thus, recorded. Following formula can be used to determine frequency.

I. Percentage of frequency (%) compartment-wise:

$$\text{Frequency (\%)} = \frac{\text{Total number of compartments in which the species occurred}}{\text{Total number of compartments studied}} \times 100$$

II. Percentage of frequency (%) Sample-wise:

$$\text{Frequency (\%)} = \frac{\text{Number of samples in which a particular fungus was recorded}}{\text{Total number of samples}} \times 100$$

(c) Abundance

It is the study of total number of individuals of different species in a community per unit area. Abundance is represented by the following equation:

$$\text{Abundance} = \frac{\text{Total number of individuals of a species in all compartments}}{\text{Number of compartments in which the species occurred}}$$

(d) Percentage of Occurrence (%)

The density and distribution of AM fungi in rhizosphere soil was expressed in terms of percentage of occurrence.

$$\text{Percentage of occurrence (\%)} = \frac{\text{Total number of individual fungus isolated}}{\text{Total number of fungi isolated}} \times 100$$

Diversity Indices

Miller *et al.* (1987) was used to analysis different diversity parameters like species richness and diversity indices like Simpson's index of diversity, Shannon-Wiener diversity index etc.

Species Richness

Total number of species recovered (T) and rhizospheric fungal species richness were determined after identifying the isolated fungus using the following formula

$$S = n + \left(\frac{n-1}{n} \right) k$$

Where, S = Species richness,

n = Total number of species present in the sample population,

k = Number of "unique" species.

Diversity Index

Shannon Diversity index was computed using the following formula:

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

Where, D = Diversity Index

N = Total number of organisms of all species found

n = Number of individuals of a particular species

Simpson's diversity indices were calculated using the formula: $S = 1/D$

Results and Discussion

The physical properties of soil collected from the sampling locations were studied (Table 1). Physical analysis of the collected soil samples revealed significant variations at all the study locations. Out of all the study locations, SBS/SOT/FS-05 showed maximum temperature of 29 °C while JRT/CHN/FS-01, JRT/BSP/FS-02 and SHG/NEHU/FS-03 respectively showed minimum temperature (20 °C). Humidity analysis showed

maximum value at study location JRT/RFRI/FS-04 (85%). It was minimum at sampling location SHG/NEHU/FS-03. The pH value of the study locations ranged from 4.5-7.05, thus representing the acidic nature of the soil samples at most of the locations. Variations were observed in electrical conductivity at all the study locations. Electrical conductivity was maximum at JRT/CHN/FS-01 (0.22 dS/m) and minimum at SHG/NEHU/FS-03 (0.08 dS/m).

Table 1. Physical properties of soil collected from the sampling location

Study site	Temperature (°C)	Humidity (%)	pH	Electrical conductivity (dS/m)
JRT/CHN/FS-01	20±0	65±3.21	7.05±0.25	0.22±0.05
JRT/BSP/FS-02	20±0	51±4.0	6±0.26	0.09±0.07
SHG/NEHU/FS-03	20±0	50±3.5	5.4±0.15	0.08±0.02
JRT/RFRI/FS-04	28±0	85±2.8	4.48±0.12	0.2±0.01
SBS/SOT/FS-05	29±0	80±3.8	4.5±0.15	0.11±0.02

* Average of three replications, mean ± SEM (standard error of the mean)

The chemical properties of soil collected from the sampling locations were also analyzed (Table 2). It is evident that, SHG/NEHU/FS-03 exhibits maximum organic carbon content (2.01%), out of all other sampling locations. Its value was minimum at SBS/SOT/FS-05 (0.7%). Available nitrogen ranged from 401±0.5 to 625±0.5 kg ha⁻¹ throughout the study locations. Maximum nitrogen content (625 kg ha⁻¹) was recorded at SHG/NEHU/FS-03 while its value was minimum at SBS/SOT/FS-05. Variations were also observed in exchangeable phosphorus throughout the study locations. Exchangeable phosphorus was maximum (40 kg ha⁻¹) at SHG/NEHU/FS-03 and JRT/BSP/FS-02 and JRT/RFRI/FS-04 respectively showed its minimum value. Available potassium was maximum at SHG/NEHU/FS-03 (650.5 kg ha⁻¹) and minimum at JRT/RFRI/FS-04 (178.18 kg ha⁻¹). Comparative analysis for soil nutrient and fertility status (NPK) showed maximum nutrient availability at SHG/NEHU/FS-03 followed by JRT/CHN/FS-01 while the other soil samples exhibited minor variations.

Table 2. Location-wise variation of chemical properties of soil

Study site	Organic carbon (%)	Available nitrogen (kg ha ⁻¹)	Exchangeable phosphorus as P ₂ O ₅ (kg ha ⁻¹)	Available potassium as K ₂ O (kg ha ⁻¹)
JRT/CHN/FS-01	0.87±0.01	457±1.3	34±1.2	408.84±1.4
JRT/BSP/FS-02	0.8±0.01	445±1.5	30±0.27	201±1.6
SHG/NEHU/FS-03	2.01±0.01	625±2.0	40±0.25	650.5±1.2
JRT/RFRI/FS-04	0.81±0.02	442±1.8	30±0.15	178.18±1.3
SBS/SOT/FS-05	0.7±0.02	401±1.6	33.6±0.10	245±1.5

* Average of three replications, mean ± SEM (standard error of the mean)

Analyses were also done to observe the altitudinal variation (Table 3 and 4) and habitat wise variation (Table 5 and 6) in the physico-chemical properties of soil.

Table 3. Elevation-wise variation of physical properties of soil

Elevations	Temperature of soil (°C)	Humidity (%)	pH	Electrical conductivity (dS/m)
Plains	24.25±0	70±3.5	5.51±0.5	0.16±0.01
Hills	20±0	50±2.0	5.4±0.5	0.08±0.01

* Average of three replications, mean ± SEM (standard error of the mean)

Table 4. Elevation-wise variation of chemical properties of soil

Elevations	Organic carbon (%)	Available nitrogen (kg ha ⁻¹)	Exchangeable phosphorus as P ₂ O ₅ (kg ha ⁻¹)	Available potassium as K ₂ O (kg ha ⁻¹)
Plains	0.80±0.01	436.25±1.5	31.90±1.0	258.26±2.3
Hills	2.01±0.01	625±1.8	40±1.2	650.5±2.5

* Average of three replications, mean ± SEM (standard error of the mean)

Table 5. Habitat-wise variation of physical properties of soil

Sl. No.	Habitats	Temperature of soil	Humidity (%)	pH	Electrical conductivity (dS/m)
1	Homestead garden	25.67±0	77±1.5	5.34±0.15	0.18±0.01
2	Riverside	20±0	51±0.5	6±0.10	0.09±0.02
3	Roadside	20±0	50±0.5	5.4±0.18	0.08±0.01

* Average of three replications, mean ± SEM (standard error of the mean)

Table 6. Habitat-wise variation of chemical properties of soil

Sl No.	Habitats	Organic carbon (%)	Available nitrogen (kg ha ⁻¹)	Exchangeable phosphorus as P ₂ O ₅ (kg ha ⁻¹)	Available potassium as K ₂ O (kg ha ⁻¹)
1	Homestead garden	0.79±0.05	433.33±1.5	32.53±0.05	277.34±1.4
2	Riverside	0.8±0.08	445±2.0	30±0.06	201±1.2
3	Roadside	2.01±0.07	625±2.7	40±0.05	650.5±1.5

* Average of three replications, mean ± SEM (standard error of the mean)

In the study, the highest pH was recorded in the samples collected from plains (5.51), as compared to that of hill region (5.4) (Table 3). Likewise in the case of electrical conductivity of soil samples, highest electrical conductivity was recorded in the plains (0.16 dS/m) followed by hilly regions (0.08 dS/m). However, very slight variations were observed in case of the temperature of soil and humidity (%) of the rhizospheric soil samples in the two elevations. Regarding the chemical properties of the soil samples (Table 2), it was observed that, the rhizospheric samples collected from the hilly region exhibited the highest amount of organic carbon content (%) (2.01), available nitrogen (625 kg ha⁻¹), exchangeable phosphorus (40 kg ha⁻¹) and available potassium (650.5 kg ha⁻¹) as compared to that of plains.

It is evident that, the highest pH was recorded in the samples collected from riverside (6), followed by roadside (5.4) and the least were observed in the homestead gardens (5.4). While reverse was in the case of electrical conductivity of soil samples. Highest electrical conductivity was recorded in the homestead gardens (0.18 dS/m) followed by roadside (0.09 dS/m) and the least was recorded in the rhizospheric samples collected from riverside (0.08 dS/m). However, very slight variations were observed in case of the temperature of soil and humidity % of the rhizospheric soil samples in the three habitats. Regarding the chemical properties of the soil samples, it was observed that, the rhizospheric samples collected from roadside exhibited the highest amount of organic carbon content (%) (2.01). Available nitrogen (625 kg ha⁻¹), Exchangeable phosphorus (40 kg ha⁻¹) and available potassium (650.5 kg ha⁻¹).

The study revealed location wise, habitat wise and elevation wise variation in the physico chemical properties of soil. This variation could be attributed to variations in topography, climate, physical weathering processes, vegetation cover, microbial activities and several other biotic and abiotic variables. Inherent soil physico-chemical properties influence the behavior of soil and hence, knowledge of soil property is important. Many soil chemical properties directly influence microbiological processes and these processes together with

soil physical-chemical processes determine (1) the capacity of soils to hold, supply and cycle nutrients including carbon and (2) the movement and availability of water.

In the present investigation, regarding the physical properties of the collected rhizospheric soil samples, it was found that, an increase in the humidity increases the percentage of organic carbon, total nitrogen and other soil nutrients. The result indicated that the chemical compositions of soils are directly related to climatic conditions. Change in other soil properties have been found to be more variable, perhaps due to differences in climate, crop rotation, soil type or length of time and soil has been under organic management.

Variations were also observed in the chemical properties of the collected rhizospheric soil samples. Soil organic carbon (SOC) is a dynamic component of terrestrial systems, with both internal changes and external exchanges with the atmosphere and the biosphere (Zhang and McGrath, 2004). SOC plays an important role in enhancing crop production and mitigating greenhouse gas emissions (Post and Kwon, 2000). Like other soil properties, SOC levels exhibit variability as a result of dynamic interactions between parent material, climate and geological history, on regional and continental scale (Wang *et al.*, 2001). However, landscape attributes including slope, aspect and elevation, and land use may be the dominant factors impacting SOC content in areas with homogeneous parent material and a single climate regime (Rezaei and Gilkes, 2005). These attributes affect organic activity, runoff and runoff processes, condition of natural drainage, and exposure of soil to wind and precipitation (Buol *et al.*, 1989).

Analyses were also done to observe the habitat wise variation and altitudinal variation in the physico-chemical properties of soil. Our study reveals variation in the physical properties of the soil. The highest soil pH, electrical conductivity and temperature were observed in the case of homestead gardens. These results are in accordance to the results obtained by John 1997, where he opined that the presence of trees lowered the soil temperature in the homestead, whereas relative humidity was maintained at an optimum level when compared to open condition. Soil pH represents a major determinant of soil microbial distribution and activity as microbes have different pH requirements (Elsas *et al.*, 2006).

Regarding the chemical properties of soil, it was observed that the rhizospheric samples collected from roadside exhibited the highest amount of organic carbon content (%) (2.01), available nitrogen (625 kg ha^{-1}), exchangeable phosphorus (400 kg ha^{-1}) and available potassium (650.5 kg ha^{-1}) i.e. soil NPK composition. The results are in contradiction to the results obtained by John (1997), where he observed an enhanced soil organic carbon content, available N, P and K status in the home gardens. The soil in the homestead had a lower bulk density, high water holding capacity, porosity and moisture content. Nutrient cycling, recycling of crop residues and addition of organic manures helped in improving and maintaining the soil physico-chemical and biological properties of the system in a sustainable manner.

In the present investigation, it was observed that the rhizospheric samples collected from the hilly region exhibited the highest amount of organic carbon content (%) (2.01), available nitrogen (625 kg ha^{-1}), exchangeable phosphorus (400 kg ha^{-1}) and available potassium (650.5 kg ha^{-1}) as compared to that of plains. These results are in accordance with the data recorded by Sevgi, 2003. Increase in the soil organic carbon is due to the change in the climatic condition at higher elevation as well the decrease in organic matter (OM) accumulation along the elevation gradients. This variation could also be attributed to the microbial activity of the soil population present in the soil samples. Soil organic matter is closely associated with microbial activity as it serves as substrates and energy sources for soil microbes (Magdoff and Weil, 2004). The change in altitudinal gradients influences soil organic matter by controlling soil water balance, soil erosion, geologic deposition processes, species and biomass production of the native vegetation and cultivated plants.

Results of NPK composition in the rhizospheric soil samples depicted an increasing trend along the altitudinal gradient. Similar observations were also made by Jha *et al.* (1992), where they observed significant correlation with the altitude gradient and that the percentage of available Zinc, Copper and Phosphorus content in the soil is increasing with the increase in altitude.

Regarding the diversity of isolated rhizospheric microfungi, a total of 46 species of fungi belonging to 19 families and 4 orders were isolated from the rhizospheric soil of *E. latifolia* (Table 7).

Table 7. Natural occurrence and diversity of mycoflora in rhizosphere of *E. latifolia* L.

Name of species	Order	Family	JRT/C HN/F S-01	JRT/B SP/FS -02	SHG/ NEHU /FS-03	JRT/R FRI/FS -04	SBS/S OT/F S-05
<i>Acremonium</i> sp.	Ascomycota	Hypocreaceae	-	-	-	-	+
<i>Aspergillus candidus</i>	Ascomycota	Trichocomaceae	-	+	-	-	-
<i>Aspergillus flavus</i>	Ascomycota	Trichocomaceae	+	+	-	-	-
<i>Aspergillus niger</i>	Ascomycota	Trichocomaceae	+	+	-	-	-
<i>Aspergillus ochraceus</i>	Ascomycota	Trichocomaceae	-	+	+	+	-
<i>Aspergillus parasiticus</i>	Ascomycota	Trichocomaceae	-	+	-	-	-
<i>Aspergillus</i> sp.	Ascomycota	Trichocomaceae	+	+	+	-	-
<i>Aspergillus terreus</i>	Ascomycota	Trichocomaceae	-	-	-	+	-
<i>Basidiobolus globose</i>	Zygomycota	Basidiobolaceae	-	-	-	-	+
<i>Botryotrichum</i> sp.	Ascomycota	Chaetomiaceae	-	-	-	+	-
<i>Chrysosporium</i> sp.	Ascomycota	Onygenaceae	-	+	-	-	-
<i>Cladosporium</i> sp.	Ascomycota	Davidiellaceae	-	-	-	-	+
<i>Corynospora</i> sp.	Ascomycota	Cornesporascaceae	-	+	-	-	-
<i>Cunninghamella</i> sp.	Zygomycota	Cunninghamellaceae	+	+	-	-	-
<i>Curvularia lunata</i>	Ascomycota	Pleosporaceae	-	-	-	+	-
<i>Curvularia</i> sp.	Ascomycota	Pleosporaceae	-	+	-	-	-
<i>Fusarium</i> sp.	Ascomycota	Nectriaceae	-	-	-	-	-
<i>Geotrichum</i> sp.	Ascomycota	Endomycetaceae	-	+	+	-	+
<i>Helminthosporium</i> sp.	Ascomycota	Pleosporaceae	-	-	-	+	-
<i>Humicola grisea</i>	Ascomycota	Chaetomiaceae	-	+	-	+	-
<i>Microsporium</i> sp.	Ascomycota	Arthrodermataceae	-	-	-	+	-
<i>Mucor racemosa</i>	Zygomycota	Mucoraceae	-	-	+	-	+
<i>Mucor</i> sp.	Zygomycota	Mucoraceae	-	+	-	-	-
<i>Nigrospora</i> sp.	Ascomycota	Trichosphaeriaceae	-	+	-	-	+
<i>Penicillium aurantiogriseum</i>	Ascomycota	Trichocomaceae	-	-	+	-	-
<i>Penicillium capsulatum</i>	Ascomycota	Trichocomaceae	+	+	-	+	-
<i>Penicillium citrinum</i>	Ascomycota	Trichocomaceae	-	+	-	+	+
<i>Penicillium commune</i>	Ascomycota	Trichocomaceae	-	+	-	-	-
<i>Penicillium crimeogriseum</i>	Ascomycota	Trichocomaceae	-	-	-	+	-
<i>Penicillium dendriticum</i>	Ascomycota	Trichocomaceae	-	+	-	-	-
<i>Penicillium oxalicum</i>	Ascomycota	Trichocomaceae	-	+	-	-	+
<i>Penicillium</i> sp.	Ascomycota	Trichocomaceae	+	-	+	-	+
<i>Pythium</i> sp.	Oomycota	Pythiaceae	+	+	-	-	+
<i>Rhizoctonia solani</i>	Basidiomycota	Ceratobasidiaceae	+	-	+	-	+
<i>Rhizoctonia</i> sp.	Basidiomycota	Ceratobasidiaceae	-	+	+	-	-
<i>Rhizomucor</i> sp.	Zygomycota	Mucoraceae	-	-	+	-	-
<i>Rhizopus</i> sp.	Zygomycota	Mucoraceae	-	-	-	-	+
<i>Sporotrichum</i> sp.	Ascomycota	Chaetomiaceae	-	-	-	+	-
<i>Sterile mycelia</i>	Ascomycota	-	-	-	-	-	+
<i>Trichoderma hamatum</i>	Ascomycota	Hypocreaceae	-	-	-	+	-
<i>Trichoderma harzianum</i>	Ascomycota	Hypocreaceae	-	-	-	+	-
<i>Trichoderma</i> sp. 1	Ascomycota	Hypocreaceae	-	-	-	+	-
<i>Trichoderma</i> sp. 2	Ascomycota	Hypocreaceae	-	-	-	+	-
<i>Trichoderma viride</i>	Ascomycota	Hypocreaceae	-	+	-	+	-
<i>Verticillium</i> sp.	Ascomycota	Plectosphaerellaceae	-	+	-	+	-
<i>Zygomycetes</i> (unknown)	Zygomycota	-	+	-	-	-	-

“+” = Present, “-” = Absent. JRT/CHN/FS-01; JRT/BSP/FS-02; SHG/NEHU/FS-03; JRT/RFR/FS-04; and SBS/SOT/FS-05 are the accession/collection numbers of the collected samples from different collection sites

The isolated species are *Acremonium* sp., *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Aspergillus* sp., *Aspergillus terreus*, *Basidiobolus globose*, *Botryotrichum* sp., *Chrysosporium* sp., *Cladosporium* sp., *Corynospora* sp., *Cunnighamella* sp., *Curvularia lunata*, *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Helminthosporium* sp., *Humicola grisea*, *Microsporium* sp., *Mucor racemosa*, *Mucor* sp., *Nigrospora* sp., *Penicillium aurantiogriseum*, *Penicillium capsulatum*, *Penicillium citrinum*, *Penicillium commune*, *Penicillium crimeogriseum*, *Penicillium dendriticum*, *Penicillium oxalicum*, *Penicillium* sp., *Pythium* sp., *Rhizoctonia solani*, *Rhizoctonia* sp., *Rhizomucor* sp., *Rhizopus* sp., *Sporotrichum* sp., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma* sp. 1, *Trichoderma* sp. 2, *Trichoderma viride*, *Verticillium* sp., *Zygomycetes* (unknown) and Sterile mycelia (**Plates 1, 2 and 3**). Ahmed *et al.* (2022) isolated several fungi from the rhizospheric soil of two types of plant by using serial dilution method. *Aspergillus flavus*, *Penicillium notatum*, *Fusarium oxysporum*, *Trichoderma harzianum*, *Penicillium chrysogenum* were isolated from the rhizosphere soil of papaya (*Carica papaya* L.). *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger* were isolated from the rhizospheric soil of eggplant (*Solanum melongena* L.). In another study by Guleri *et al.* (2021), they isolated around thirty-four species of microfungi belonging to 21 genera from Garhwal Himalaya. *Cladosporium* was the most dominant genus, followed by *Aspergillus* and *Fusarium*. *Cladosporium cladosporioides* was the most common fungi isolated from *Hippophae* growing sites with maximum contribution (7.43%) whereas, *Nigrospora sphaerica* was the least important species with minimum contribution (0.80%). The highest number of species (23) was isolated from the Mana site of Chamoli District of Garhwal Himalaya. In a study conducted by Abbasi *et al.* (2021), 20 taxonomic ranks were diagnosed, and Deuteromycota outperformed all the isolates, as it recorded nine taxonomic ranks, including five of them belonging to the genus *Aspergillus* and four of them to the genus *Penicillium*, followed by *Ascomycota*, which displayed five taxonomic ranks, two ranks belong to the genus *Alternaria* and three to the genus *Alternaria*.

The highest density (0.5) was observed in case of *Basidiobolus globose*, *Corynospora* sp., *Microsporium* sp., *Sporotrichum* sp. and *Trichoderma viride* and lowest (0.13) in *Acremonium* sp., *Aspergillus niger*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Botryotrichum* sp., *Cunnighamella* sp., *Curvularia lunata*, *Fusarium* sp., *Humicola grisea*, *Mucor* sp., *Nigrospora* sp., *Penicillium aurantiogriseum*, *Penicillium capsulatum*, *Penicillium commune*, *Penicillium dendriticum*, *Penicillium oxalicum*, *Pythium* sp., *Rhizoctonia solani*, *Rhizoctonia* sp., *Trichoderma hamatum*, *Trichoderma* sp. (2) and *Zygomycetes* (unknown) (Figure 2).

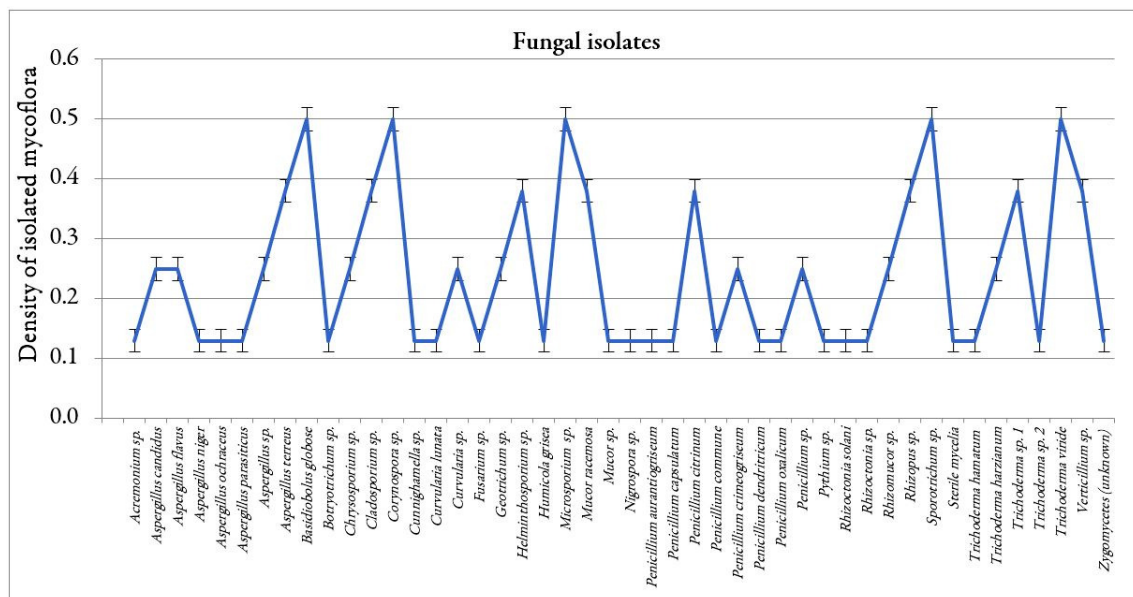


Figure 2. Density of the isolated mycoflora

Natural occurrence of fungi showed highest (1.0%) location/region-wise frequency in case of *Aspergillus ochraceus* (Figure 3), followed by *Aspergillus flavus*, *Aspergillus niger*, *Chrysosporium* sp., *Cunninghamella* sp., *Curvularia lunata*, *Geotrichum* sp., *Penicillium capsulatum*, *Penicillium* sp., *Pythium* sp., *Rhizoctonia solani* and *Rhizopus* sp.

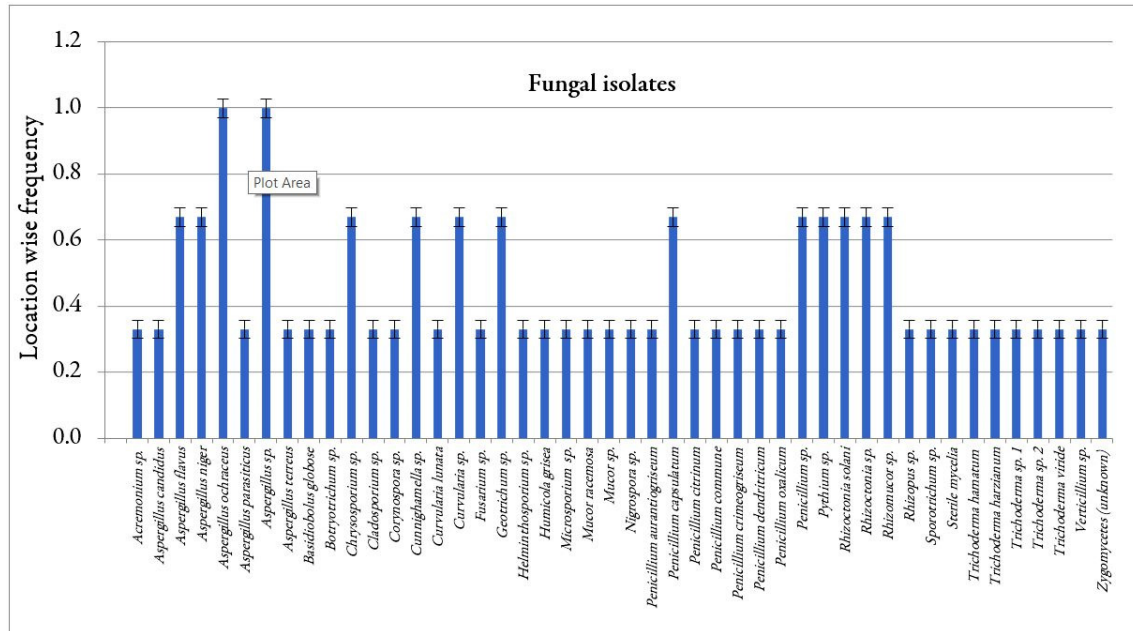


Figure 3. Location wise frequency of the fungal isolates

The highest abundance value (4.0) was observed in *Rhizoctonia solani* followed by *Aspergillus candidus* (3.0) and the lowest was observed in *Rhizoctonia* sp. (0.5) (Figure 4).

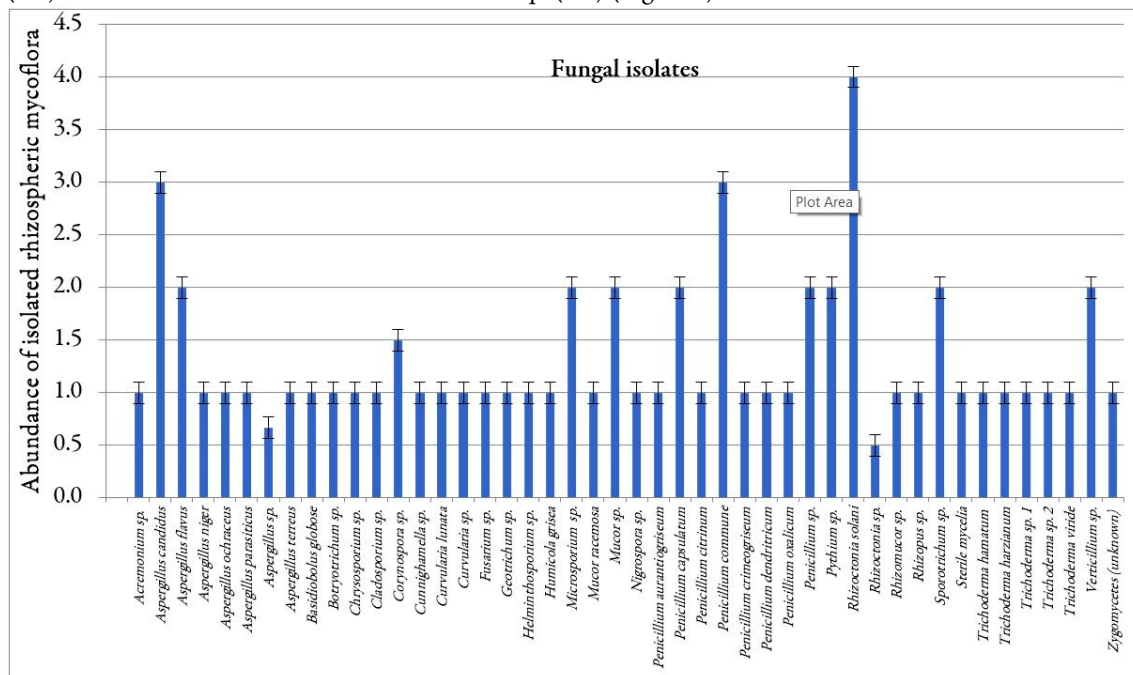


Figure 4. Abundance of isolated mycoflora

In contrary to region wise frequency as cited above, the percent natural occurrence and species-wise frequency (Figure 5 and Figure 6) were highest in *Aspergillus ochraceus* and *Aspergillus* sp. (7% and 60% each) respectively while lowest for *Acremonium* sp., *Aspergillus candidus*, *Aspergillus parasiticus*, *Aspergillus terreus*, *Basidiobolus globose*, *Botryotrichum* sp., *Cladosporium* sp., *Corynospora* sp., *Cunninghamella* sp., *Curvularia lunata*, *Fusarium* sp., *Helminthosporium* sp., *Humicola grisea*, *Microsporium* sp., *Mucor racemosa*, *Mucor* sp., *Nigrospora* sp., *Penicillium aurantiogriseum*, *Penicillium citrinum*, *Penicillium commune*, *Penicillium crimeogriseum*, *Penicillium dendriticum*, *Penicillium oxalicum*, *Rhizopus* sp., *Sporotrichum* sp., *Trichoderma hamatum*, *Trichoderma harzianum*, *Trichoderma* sp., *Trichoderma viride* and *Verticillium* sp. (2% and 20% each) respectively.

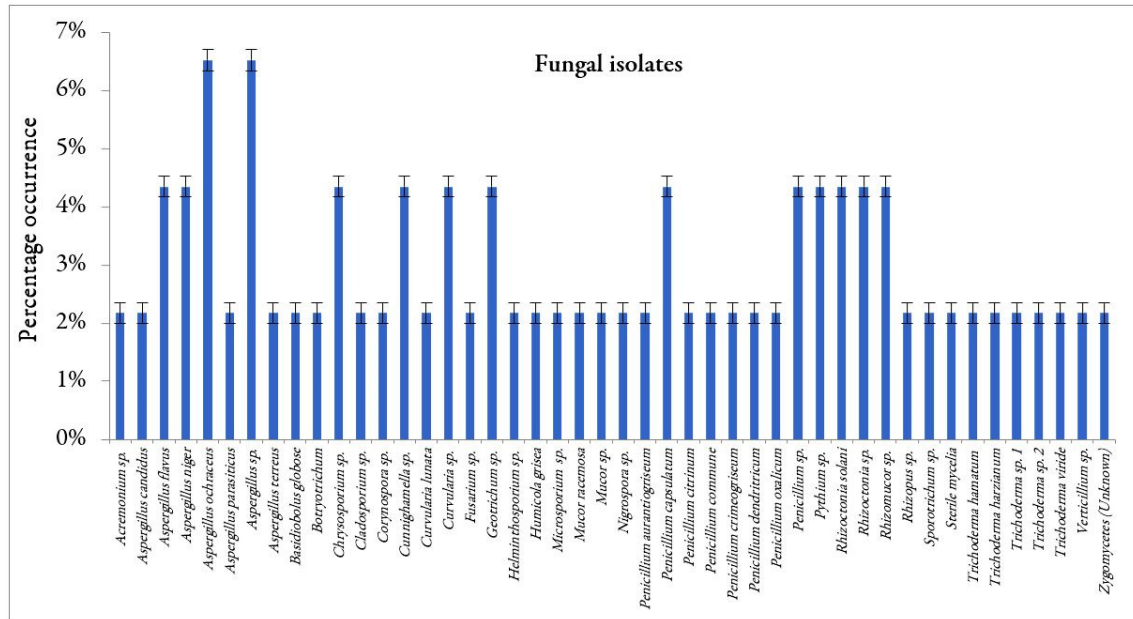


Figure 5. Occurrence (%) of the isolated rhizospheric mycoflora

Figure 7 depicts the generic diversity of the isolated rhizospheric mycoflora. From the figure it is evident that *Penicillium* sp. (17.39%) and *Aspergillus* sp. (15.20%) are the predominant fungal species followed by *Trichoderma* sp. (10.87%). In a similar study by Tchan *et al.* (2023), they found that soil fungal communities were host specific ($P = 0.001$). Basidiomycota were more frequently observed in the rhizosphere of Fabaceae, except for *I. doka*, whereas Ascomycota are more abundant in the rhizosphere of Phyllanthaceae (*U. togoensis*) and Dipterocarpaceae (*M. kerstingii*). The genus *Sebacina* is predominantly linked to *M. kerstingii* and *I. tomentosa*, while *Russula* is dominant under *B. grandiflora* and, *Inocybe* with *I. tomentosa*.

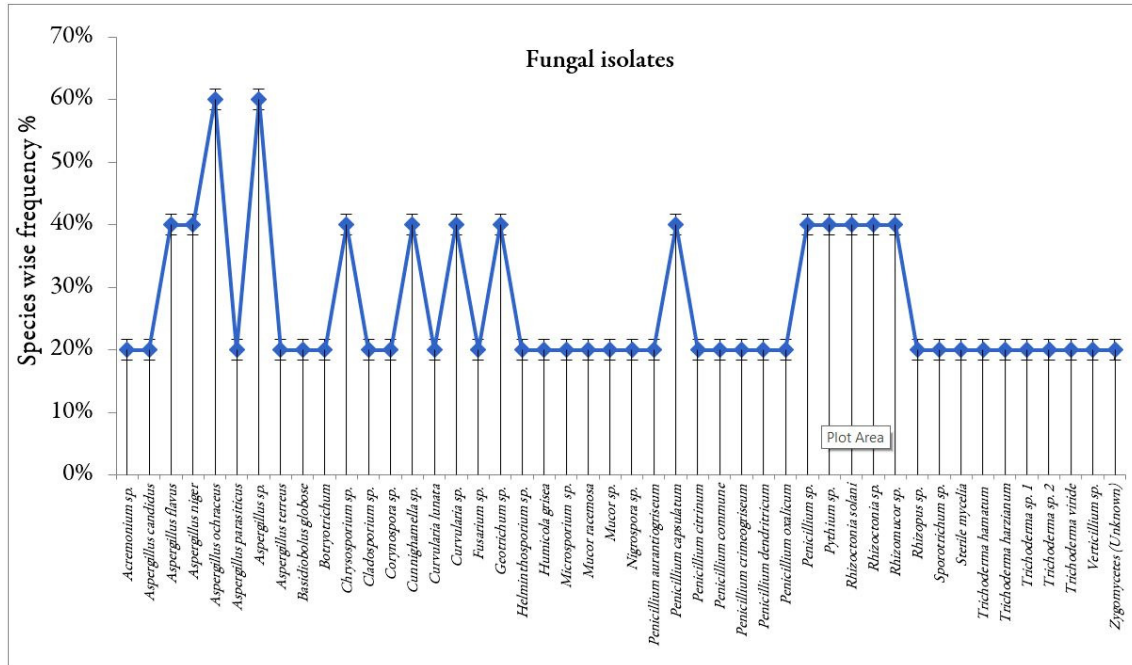


Figure 6. Species-wise frequency (%) of the isolated rhizospheric mycoflora

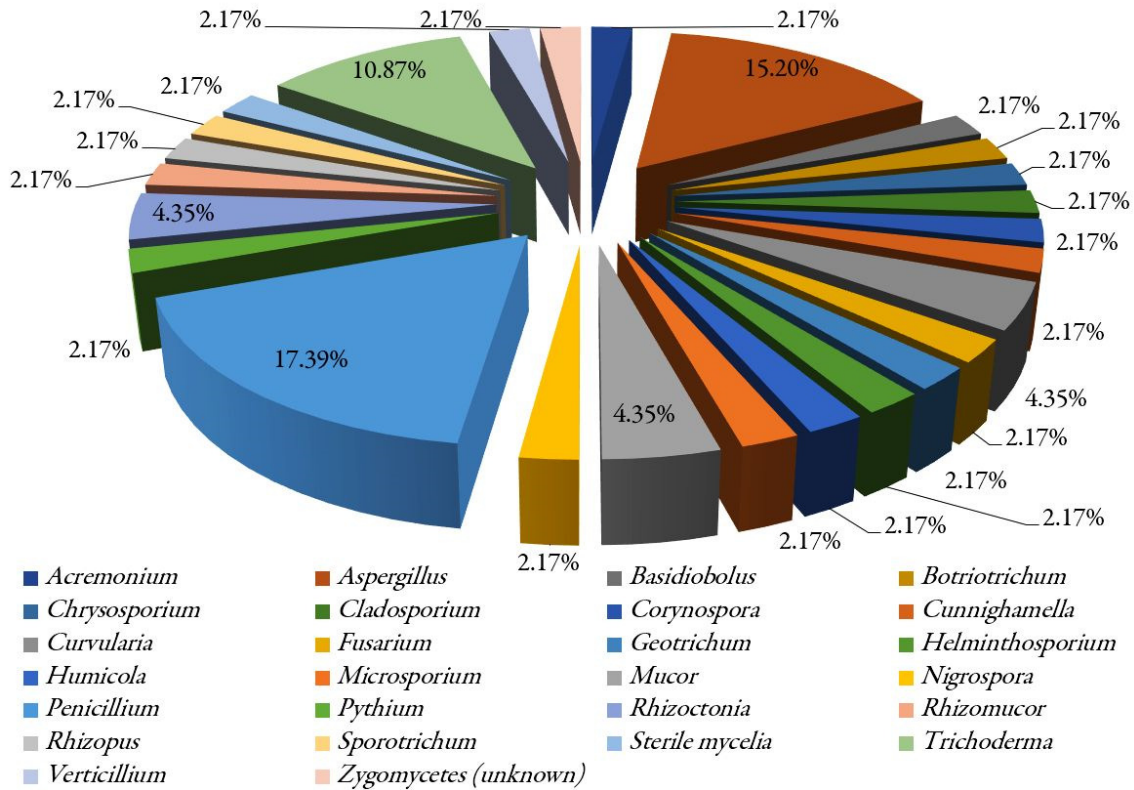


Figure 7. Generic diversity of isolated mycoflora

Location wise variation in the natural occurrence of mycoflora in the collected rhizospheric samples of *E. latifolia* was also observed (Figure 8). From the histogram it is evident that the highest percentage of occurrence of mycoflora is availed by JRT/BSP/FS-02 (50%) followed by JRT/RFRI/FS-04 (37%) and the least was found in JRT/CHN/FS-01 and SHG/NEHU/FS-03 (20% each) respectively. It was evident that maximum number of fungal isolates were found in homestead garden (70%), followed by that in the riverside (50%) and least in the roadside (20%) (Figure 9).

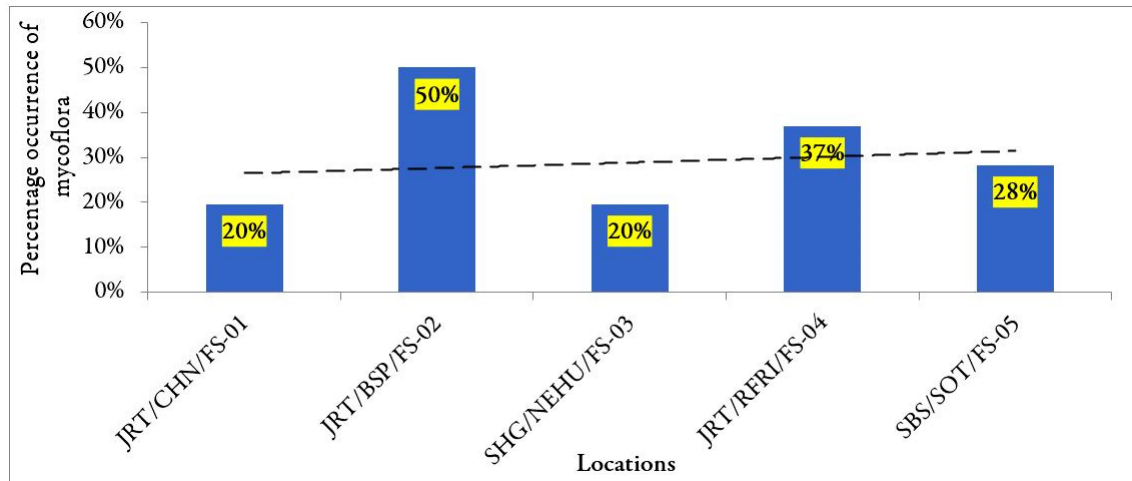


Figure 8. Location wise natural occurrence of mycoflora in the collected soil samples of *E. latifolia* L.

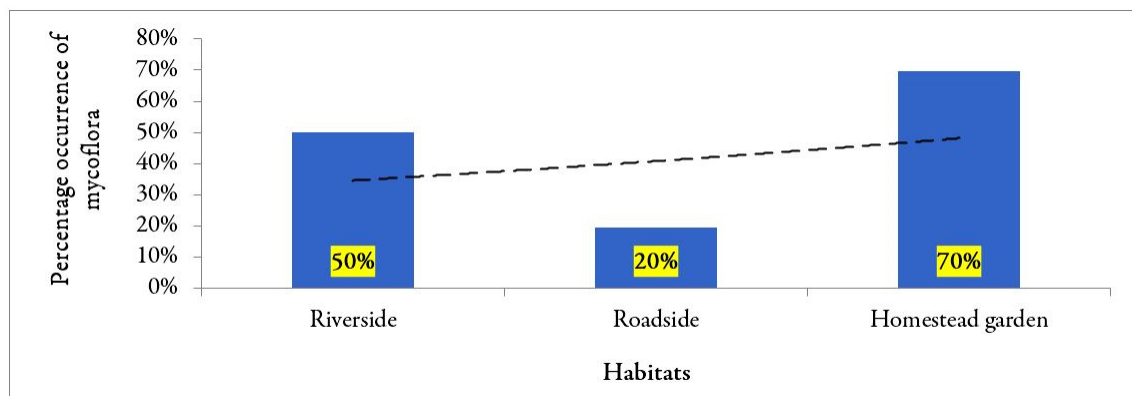


Figure 9. Habitat wise natural occurrence of mycoflora in the collected soil samples of *E. latifolia* L.

Elevation wise natural occurrence and diversity of mycoflora in the rhizosphere of the target plant species were also observed (Figure 10). It is evident from the figures that maximum number of fungal isolates was found in plains (67%) as compared to that of the rhizospheric soil samples collected from the hilly region (33%).

Significant variations were also observed related to the species richness and diversity index (Figure 11, 12 and 13). The study of species richness and diversity index showed that maximum species richness value (36.39) was observed in JRT/BSP/FS-02 whereas minimum species richness (13.44) was observed in SHG/NEHU/FS-03 location. Again, the highest Shannon diversity index value was observed in JRT/BSP/FS-02 (3.035) followed by SBS/SOT/FS-05 (2.507) and the least was observed in SHG/NEHU/FS-03 (2.107). Likewise, the highest Simpson diversity index value was observed in JRT/BSP/FS-02 (0.947) followed by JRT/RFRI/FS-04 (0.93) and the least was observed in JRT/CHN/FS-01 (0.875).

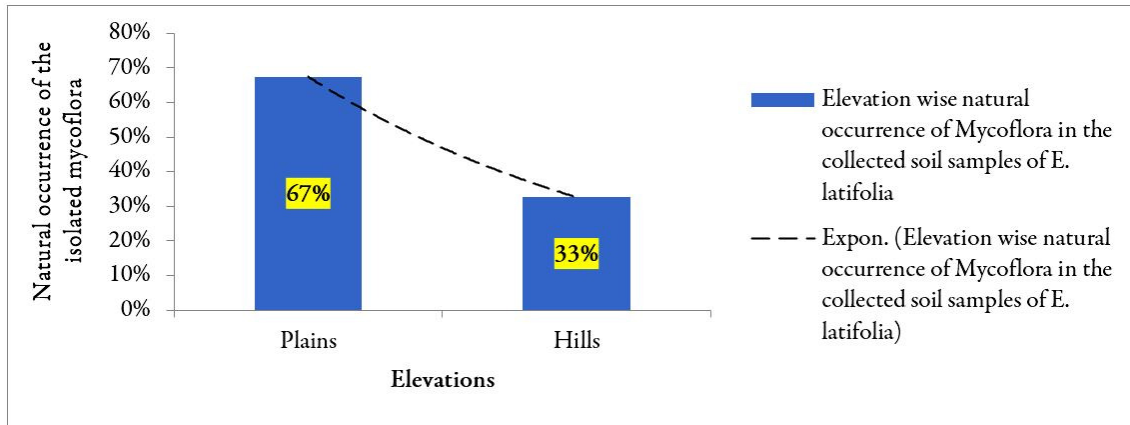


Figure 10. Elevation wise natural occurrence of mycoflora in the collected soil samples of *E. latifolia* L.

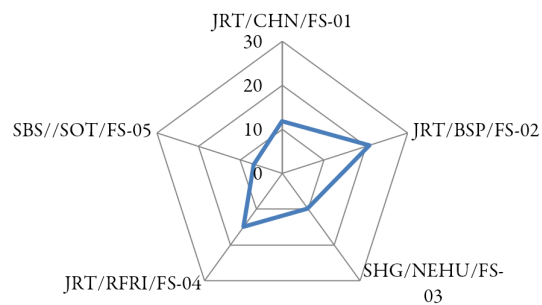


Figure 11. Species richness of the isolated rhizospheric mycoflora

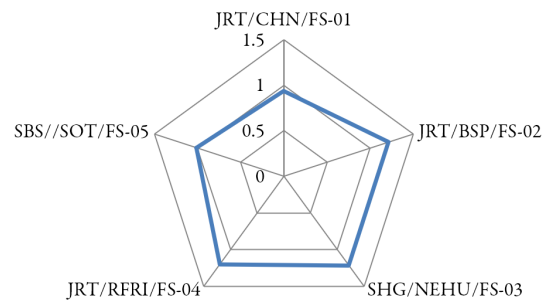


Figure 12. Shannon diversity index of the isolated rhizospheric mycoflora (on an average data)

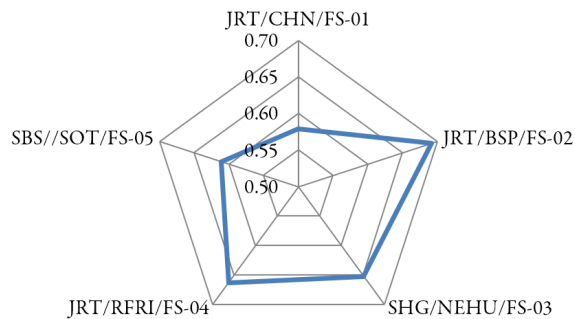


Figure 13. Simpson Diversity Index of the isolated rhizospheric mycoflora (on an average data)

A total of 46 species of fungi belonging to 19 families and 4 orders were isolated from the rhizospheric soil of *E. latifolia*. A similar investigation on rhizospheric 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 *Aspergillus* 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 (Herrera *et al.*, 2010), since they are intimately associated with diverse biological processes like organic matter decomposition, recycling and transportation of nutrients, degradation of xenobiotics compounds etc.

The study locations also showed marked variation in fungal species composition. *Basidiobolus globose*, *Corynospora* sp., *Microsporium* sp., *Sporotrichum* sp. and *Trichoderma viride* 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* sp. and *Mucor* sp. 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.

Elevation wise natural occurrence and diversity of mycoflora in rhizosphere of the target plant species shows that maximum number of fungal isolates was found in plains (67%) as compared to that of the rhizospheric soil samples collected from the hilly region (33%). Similar observations were made in quantitative species composition in both forest stands at the higher altitude, but at the lower altitude slight variations were observed between stands (Jha *et al.*, 1992). This can be attributed to the change in vegetational cover at the sites and to variations in the nutrient status of the soils (Wohlrab *et al.*, 1963). Habitat wise natural occurrence and diversity of mycoflora in rhizosphere of *E. latifolia* L. was maximum number for homestead garden (70%), followed by that in the riverside (50%) and least in the roadside (20%). Diversity analysis for soil fungi isolated from different land use systems of North Brahmaputra Valley, Assam was also made by Bhattacharyya (2012), thus, indicating a significant diversity pattern for isolated fungal strains. More diverse fungal isolates and high species richness at sampling points might be attributed to the presence of more organic nutrients in soil (Bjorbaekmo and Carlsen, 2010).

Conclusion

In the rhizosphere of medicinal plants, fungal colonization, abundance, and dispersion vary and can be influenced by a variety of factors, including the type of host plant, the time of year, the characteristics of the soil, the local climate, and environmental conditions. Microbial diversity analysis of the mycofungal population revealed that the target plant species harbors a variability of fungal population which plays a part and parcel role in the growth and development of the species. Study on the physico-chemical properties of the soil revealed that there are lots of variation among the soil samples owing to the topography, climatic conditions and altitudinal variations. The study revealed that a large number of mycobiota are associated with the species and variations were observed in the distribution pattern of the mycobiota in correlation to the location wise, habitat wise and elevation wise allocation of the target plant species. Moreover, it can be speculated that the beneficial mycobiota isolated from the plant species must be explored for further potential applications as bioagents, biofertilizers and biopesticides for a sustainable agriculture.

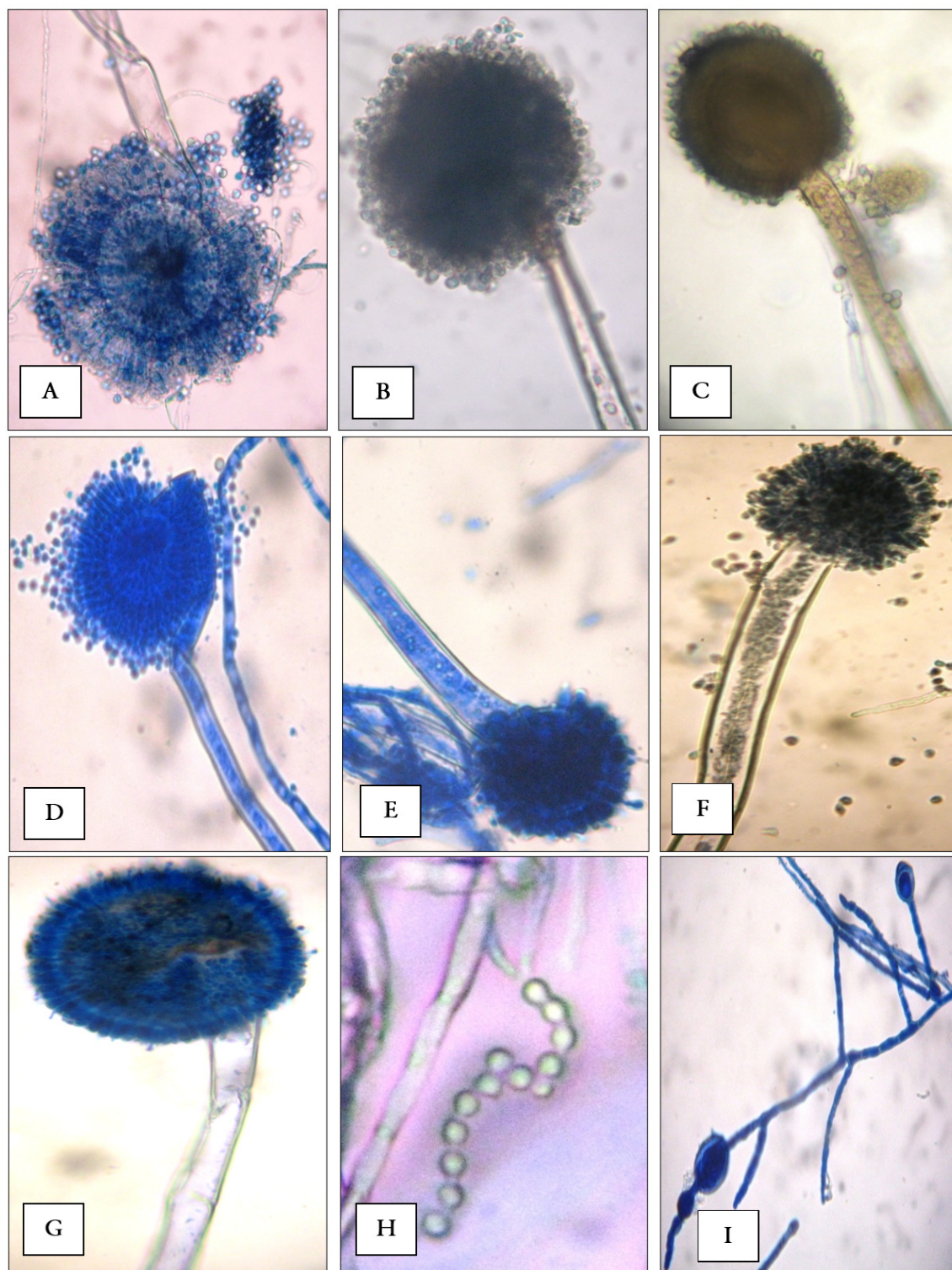


Plate 1. Some of the isolated mycofungal species (at 400X): A) *Aspergillus flavus*; B) *A. nigrum*; C) *A. ochraceus*; D) *Aspergillus* sp.; E) *A. parasiticus*; F) *A. candidus*; G) *A. ochraceus*; H) *Chrysosporium* sp.; I) *Cladosporium* sp.

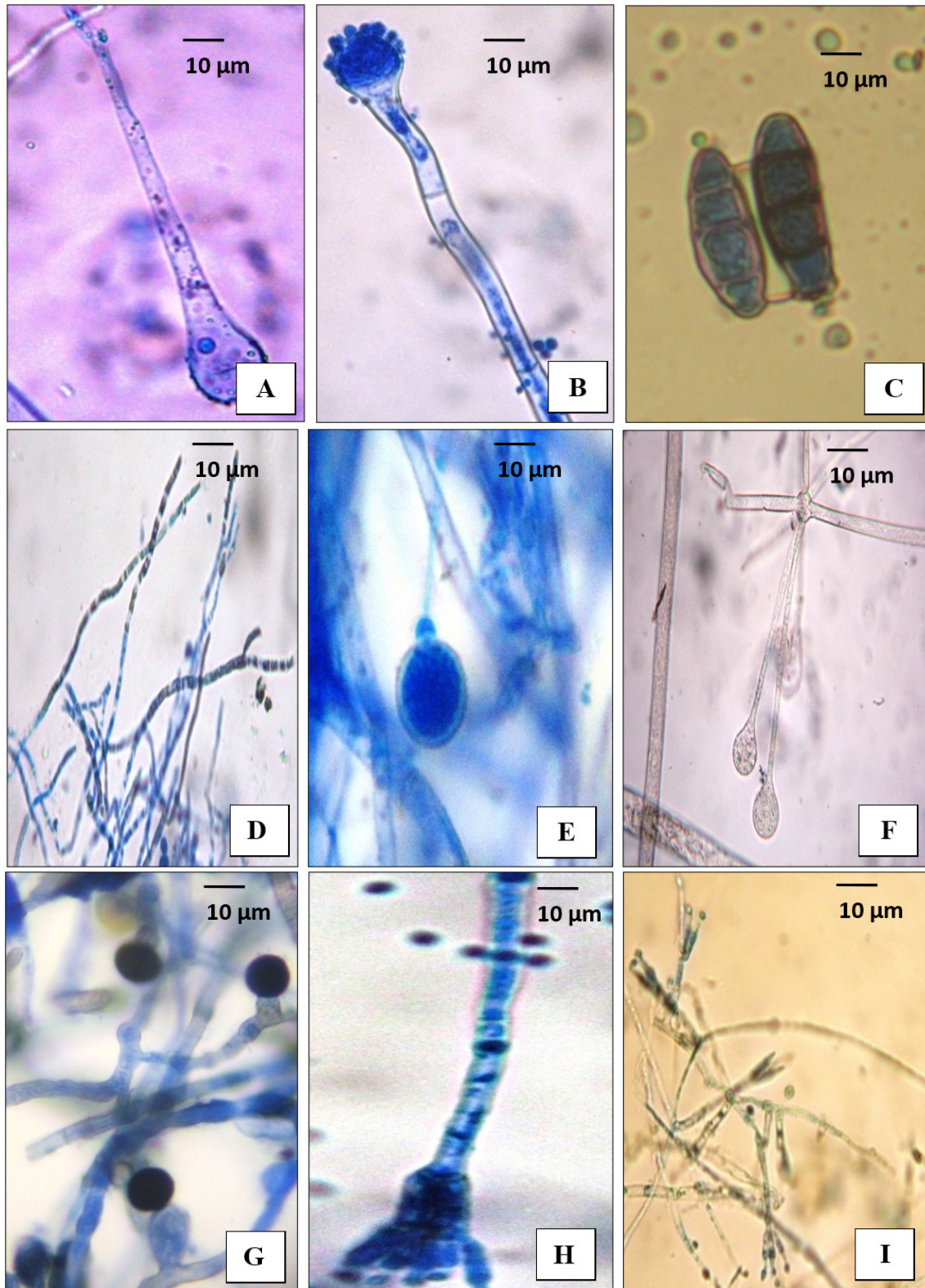


Plate 2. Fungal species isolated from the rhizosphere of *E. latifolia* L. (at 400X): A) *Cunninghamella* sp.; B) *Cunninghamella* sp.; C) *Curvularia lunata*; D) *Geotrichum* sp.; E) *Humicola grisea*; F) *Mucor racemose*; G) *Nigrospora* sp.; H) *Penicillium capsulatum*; I) *Penicillium dendriticum*

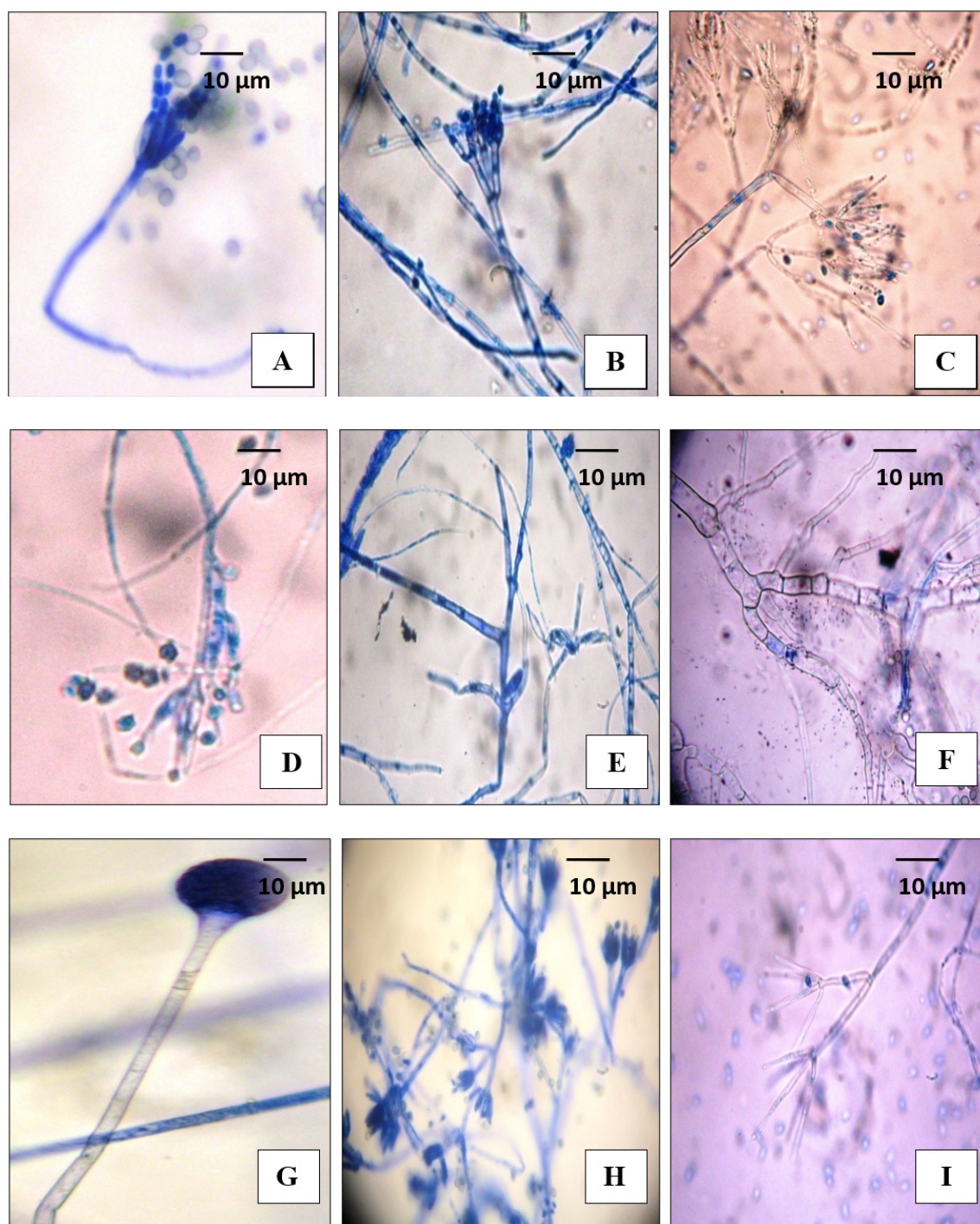


Plate 3. Fungal species isolated from the rhizosphere of *E. latifolia* L. (at 400 X): A) *Penicillium capsulatum*; B) *P. capsulatum*; C). *P. commune*; D) *P. oxalicum*; E) *Pythium* sp.; F) *Rhizoctonia solani*; G) *Rhizomucor* sp.; H) *Trichoderma viride*; I) *Verticillium* sp.

Authors' Contributions

Equal contributions. The authors read and approved the final manuscript.

Ethical approval

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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