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A comparative analysis of different rhizospheric soil mycoflora in Gibbon wildlife sanctuary and its nearby area, Assam, India

Dipankar Gogoi^{1*}, Nazim Forid Islam², S. C. Rajkhowa², Harajyoti Mazumdar³ and Achyut Bhagowati¹

¹Institutional Biotech Hub, N. N. Saikia College, Titabar

²Department of Botany, N. N. Saikia College, Titabar

³The Energy and Resource Institute, North-Eastern Region, Guwahati

ABSTRACT

Fungal communities associate with roots play an important role in nutrient cycle, supporting plant growth and the biocontrol of plant diseases. This paper has evaluated the rhizospheric soil mycoflora in the Gibbon wildlife sanctuary and its surrounding area and a comparative study between these locations. Identification and characterization was done by the help of standard protocols. All total 16 nos. of fungal species have observed during the studies. The *Aspergillus* shows as the dominant mycoflora in both the location. Four species have found in *Aspergillus* group i.e. *A. niger*, *A. fumigatus*, *A. varicolor* and *A. clavatus*. In all locations same fungal species were found, but vary in their percentage of occurrence. Other major mycoflora species found in this study were *Penicillium chrysogenum*, *P. notatum*, *Rhizopus nigricans*, and *R. stolonifer*.

Keywords: Biocontrol, Rhizosphere, Gibbon wildlife sanctuary, Species, Mycoflora.

INTRODUCTION

The soil is highly complex and a favourable habitat for microorganisms and it is a suitable environment that supports extremely diverse communities of micro and macro organisms. Soil is often considered as “Black Box” [1]. It is a natural culture media for growth of microorganisms. Maximum microbial growth and activity in the soil is confined around the root systems of plants and this region is called the rhizosphere. Our study is mainly focus on determination of the rhizospheric mycoflora of soil.

The size of the microbial population in soil is of ecological importance, because of the essential role that microorganisms play in the conservation and cycling of plant nutrients [2]. The most important function of soil microorganisms is to decompose various kinds of organic matters. Amongst the microorganisms fungi play an important role on decaying of plants, so the study on fungi is going to be significant one. On the other hand, fungi are equally known for their pathogenic nature [3, 4]. They cause diseases of the root or stem disrupting the uptake and translocation of water and nutrients from the soil. Therefore, these commonly cause similar symptoms to drought and nutrient deficiencies; these include wilting, yellowing, stunting and death of plant [5].

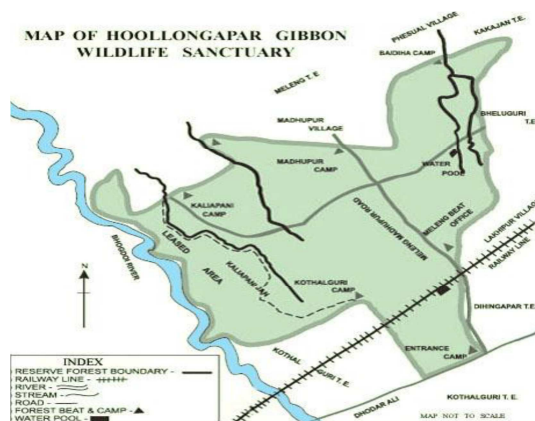
However it has been shown that fungi play pivotal role in decomposing the plant material. Many fungal species cause “white rot” of wood decay in which the wood becomes a bleached appearance, with a spongy, stringy, or laminated structure, and where lignin as well as cellulose and hemicelluloses are broken down [6]. For example the Genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Alternaria* etc., play a role as decomposers of cellulosic

materials of plants. On the other hand Hemicellulose is degraded by all major groups of fungi - most important ones are the species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium* etc. Pectic substances are degraded by species *Rhizopus*, *Aspergillus*, *Penicillium*, *Fusarium* etc. From all these sources it has been clarified that fungi is not responsible for the growth of plants but also act as a good decomposer [8].

For the study of soil mycoflora we have taken the area at Gibbon wild life sanctuary. The Gibbon wildlife sanctuary known as the Hoollongapar Gibbon Wildlife Sanctuary, a well known reserve forest in India, with the best example of conservation strategy and well growth forms of plants. This sanctuary is the only habitat in India for the species of apes, popularly known as Hoolock gibbon. The sanctuary was officially constituted and renamed in 1997 [11, 12]. It was named after its dominant tree species, *Hollong* or *Dipterocarpus macrocarpus*. In this investigation we observed that the rhizospheric soil mycoflora of four selected plant which is related to Hoolock gibbon inside the dense forest and outside the forest and show up a comparative view of mycoflora between this two groups of location. Recent studies have pointed out the ecology of soil mycoflora and its importance in growing of plants and screening of soil mycoflora.

MATERIALS AND METHODS

The main purpose of this present research is to compare the different fungal species present in selected plant species in Gibbon wildlife sanctuary and its outside area. The Gibbon Wild Life Sanctuary is located in the Jorhat district of Assam (India). It is situated in close proximity to the Naga Hills and the town of Mariani. Its geographical location is 26°40'N to 26°45'N latitude and 94°20'E to 94°25'E longitude [11-13].



Basic works begin with collecting selected plant species situated in both inside and outside the sanctuary. The plant species are Hoolong (*Dipterocarpus macrocarpus*), Dimoru (*Ficus glomerata*), Magnoliaceae (*Talauma hodgsoni*) and Seleng (*Sapium baceatum*).

The rhizosphere soil samples of these plants were collected from September 2014 to December 2014. Soil samples were collected from the depth of 5-10 and 10-15 cm in sterilized polyethylene bags and stored at 4°C in the laboratory until the examination.

Soil PH was determined by an electrical digital PH meter in a 1: 5(w/v) soil-water suspension. 5g soil sample was taken into labelled 50 ml plastic tubes to which 25 ml of distilled water was added. The soil-water suspension was shaken for 1hr using the mechanical shaker. The soil was allowed to settle for a few minutes and the pH was measured after a two point (pH 4 and pH 7) calibration of the pH meter.

On other hand the Soil moisture was estimated by Gravimetric method. 100 g of fresh soil sample was taken in an aluminium moisture box and kept in the oven at 105°C for about 24 hr until all the moisture is driven off. After removing from oven, they are cooled to room temperature and weighed again. The difference in weight was considered to be moisture contained in 100 g soil sample. The percentage moisture content was calculated as follows-

$$\text{Moisture (\%)} = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100$$

Isolation and identification of fungi

Isolation of fungi was performed by making serial dilution of the collected soil samples and the dilution was used for studies between 10^{-3} and 10^{-5} . The dilution was spread in PDA medium where 1% of Streptomycin solution was added for preventing bacterial growth. The plates were then incubated at $27 \pm 1^\circ\text{C}$. Fungal colonies were counted on the 7th day of incubation [14].

The CFU was calculated and the percentage frequency of occurrence for each species of fungus isolated was determined by the following formula:

$$\% \text{ of occurrence} = \frac{\text{Total no. of CFU of an individual sp.}}{\text{Total no. of CFU of all sp.}} \times 100$$

Identification was done with the help of standard literature study by observing colony feature fungal morphology and by microscopically staining with lactophenol cotton blue [15, 16].

RESULT AND DISCUSSION

This paper classified the fungal diversity among selected plant species taken from different location of Gibbon wild life sanctuary and its nearby location. Fungal diversity is significantly differed across the both locations. It is found from our observation that many of the fungal species were mostly found in the dense forest in comparison to outsider location. Different factors are involved in growth of fungi such as pH, soil texture, and moisture content and environmental. The pH found range between 6.35 to 7.42 in both the locations, and the range of moisture content in both the locations is found in between 14.15% and 16.46%.

The maximum fungal species belongs to Aspergillus species (i.e. *A. varicolor* 2.65%, *A. niger* 7.9%, *A. fumigatus* 4.4%, *A. clavatus* 6.19%) found in dense forest whereas less amount of same species were found in outside location (i.e. 2.1% of *A. varicolor*, 4.2% of *A. niger*, 4.2% of *A. clavatus*) except *A. fumigatus* which has observed the highest number of % of occurrence i.e., 7.4%. The result indicate the genus Aspergillus appeared as a dominant genus in all four groups of rhizospheric soil sample in both location proving the high adaptability of the genus and the second dominant species are Penicillium sp. (% of occurrence of *P. chrysogenum* and *P. notatum* in dense forest are 2.12% & 3.5% respectively whereas in outside area it is observed as 2.12% & 3.19% respectively.) and Rhizopus sp. (*R. nigricans* 5.3% and *R. stolinifer* 6.19% in dense forest and 2.1% and 3.1% in outside area).

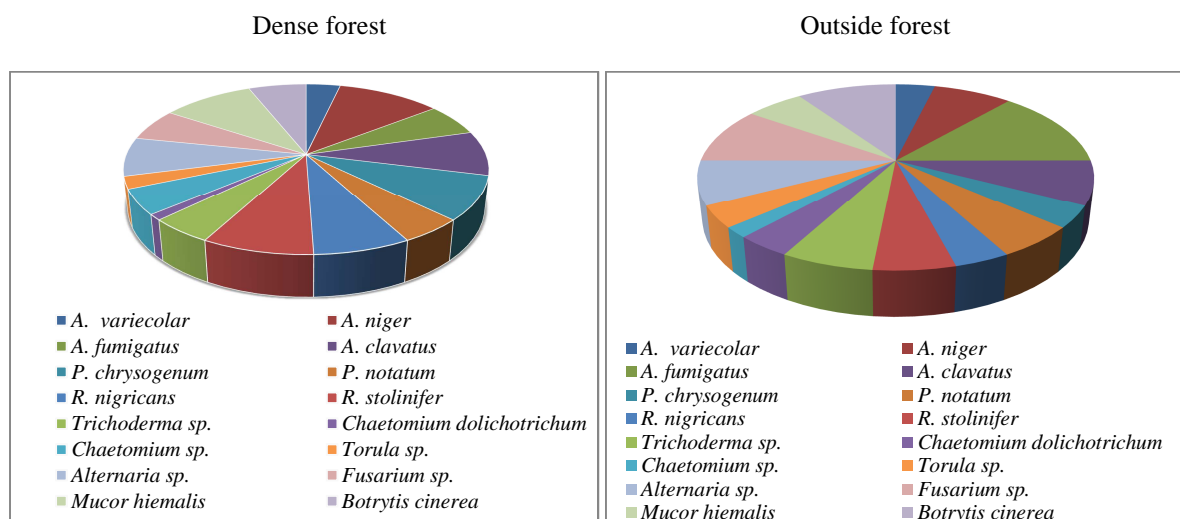


Fig 1: Comparative analysis of different fungal species occurred in both dense forest and outside forest.

However among the other fungal species Trichoderma found in greater outside area in comparison to the dense forest, i.e. 5.3% and 3.5% respectively. Other observed fungal species are Chaetomium sp. 3.5%, *Chaetomium dolichotrichum* 0.88%, *Torula sp.* 1.76%, *Alternaria sp.* 5.3%, *Fusarium sp.* 4.4%, *Mucor hiemalis* 7.0% and *Botrytis cinerea* 4.4%, in the dense forest whereas % of occurrence of the same species were observed in outside area are 1.06%, 2.1%, 2.1%, 4.2%, 5.3%, 3.1% & 5.3%, respectively.

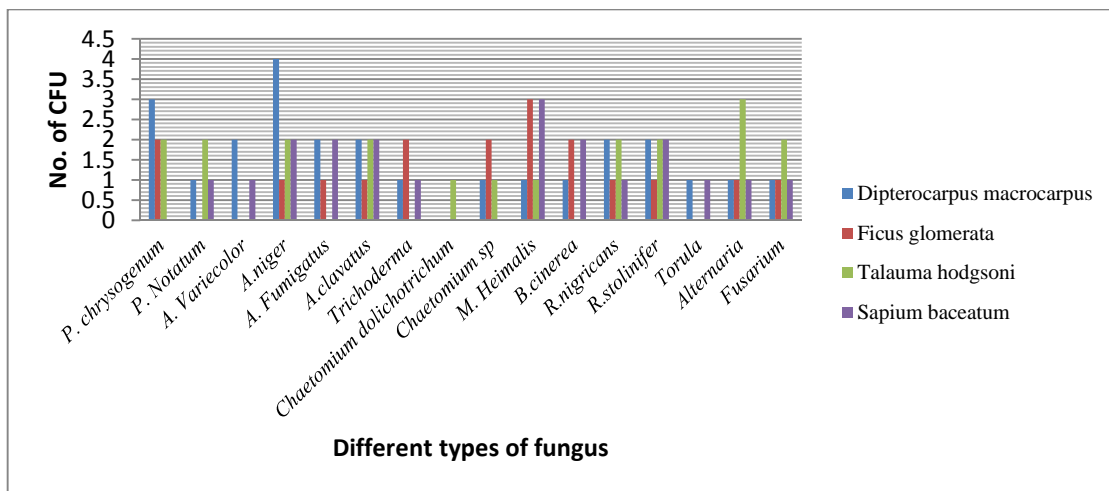


Fig 2: Fungal Sp. found in different Rhizospheric soil sample in dense forest.

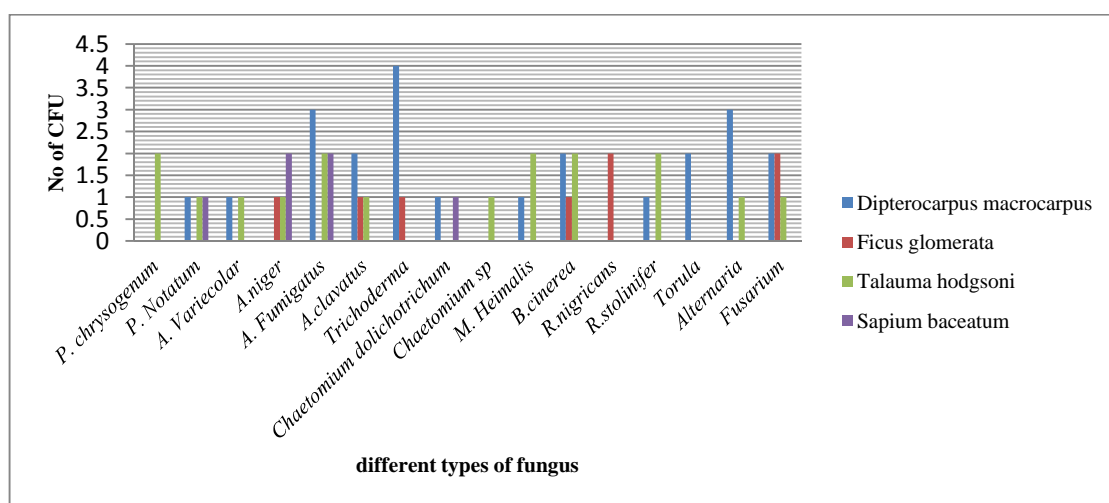


Fig.3: Fungal Sp. found in different Rhizospheric soil sample in outside forest.

Different fungal sp. show variable in growth by the different source of rhizospheric soil sample and another fact of growth is the different sites. Herein we have shown the variability of fungus in different site of locations as in Fig2 & 3. Soil samples of plant sources like *Dipterocarpus macrocarpus*, *Talauma hodgsoni* show good growth of fungus in both the location whereas soil samples of *Ficus glomerata* & *Sapium baceatum* show better fungus growth in dense forest in comparison to outside forest.

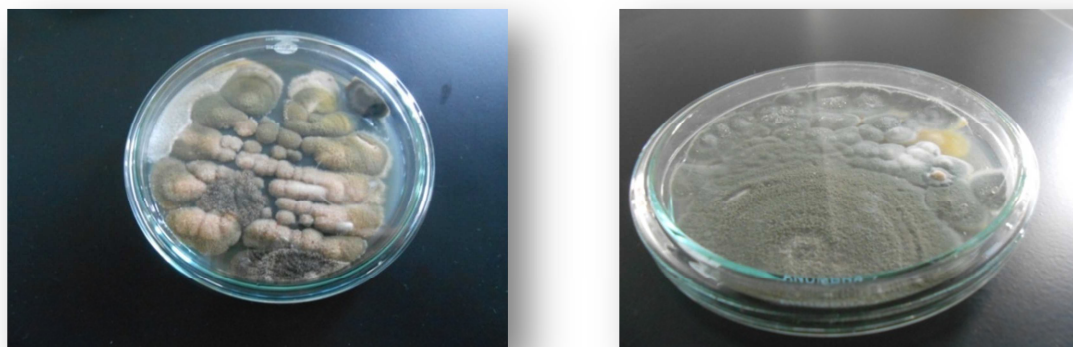
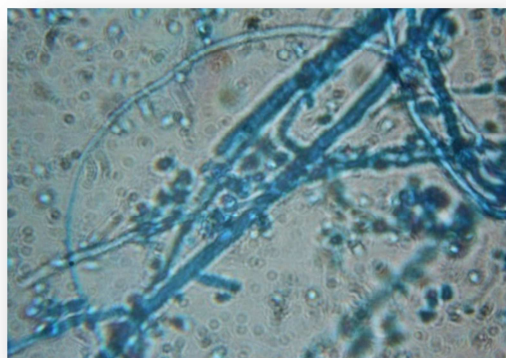


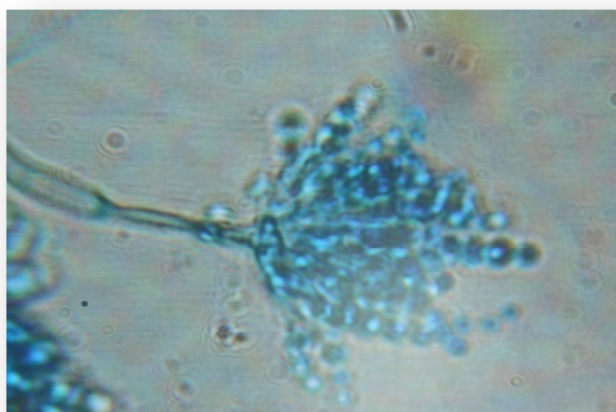
Fig.4: Fungal growth on PDA media



[i]



[ii]



[iii]

Fig.5: Microscopic view of some fungal species,[i]. Trichoderma sp.,[ii].Aspergillus sp. & [iii]Penicillium sp.

CONCLUSION

The study notified that variation of fungal species have in everywhere site by site. This study showed that the majority of Aspergillus species were found in both the locations and % of occurrence is also varying in site wise. It may be due to environmental factor such as moisture, pH etc. We have taken only four groups of rhizospheric soil sample and from this a little amount of fungal sp. were identified. The relationship between biodiversity of soil fungi and ecosystem function is an issue of paramount importance, particularly in the face of global climate change and human alteration of ecosystem processes. The periodicity occurrence of different fungal species fluctuated due to ecological and biological factors of the soil. The above study shows a comparative analysis of Different Rhizospheric soil mycoflora in Gibbon wildlife sanctuary and its nearby area, Assam, India. By this study we are trying to screened out the rhizospheric mycoflora and give awareness about the soil conservation and maintain the ecological balance.

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