Int. J. Adv. Res. Biol. Sci. 2(10): (2015): 96–102

International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

Fungal Diversity in the Rhizosphere of *Nepenthes khasiana* Hook. f., an Endemic and Endangered Insectivorous Plant of Meghalaya, India

Sophie Phanri Majaw[1], Ksanbok Makdoh[2*], Haobam Romola Devi[3] and Highland Kayang[4]

[1, 3 and 4]Centre for Advance Studies in Botany, North-Eastern Hill University, Shillong, Meghalaya, INDIA
[2]Department of Botany, Lady Keane College, Shillong, Meghalaya, INDIA
*Corresponding author: *ksanbok@gmail.com*

Abstract

Fungal diversity in the rhizosphere of *Nepenthes khasiana* Hook f. was studied from its natural habitat in coal mining areas of Khliehriat, East Jaintia Hills District, Meghalaya, India. The study was conducted during winter and pre-monsoon period from January to April 2013. Colony Forming Unit of fungi (CFU) ranges from $11.9x10^3g^{-1}$ dry weight of soil in January to $25x10^3g^{-1}$ dry weight of soil in March. A total number of 26 fungal species were isolated where *Cladosporium sphaerospernum, Penicillium janthinellum, P. brevicompactum* and *P. frequentans* were the most abundant species. *Penicillium* was found to be the most common and dominant genera. Shannon diversity index was highest in February and lowest in March whereas, Simpson dominance index was highest in March and lowest in February. CFU of fungi was positively correlated with soil temperature, moisture content, pH, organic carbon and exchangeable potassium. The present study highlights for the first time the diversity of rhizosphere mycoflora of *N. khasiana*.

Keywords: Rhizosphere mycoflora, *Nepenthes khasiana*, Endangered species.

Introduction

The rhizosphere can be defined as the zone of soil around plant roots whereby soil properties are influenced by the presence and activity of the root. It is a complex environment where roots interact with physical, chemical and biological properties of soil through a range of mechanisms, which include acidification through proton extrusion and the release of root exudates. Any changes in the properties of the rhizosphere have significant influence on the subsequent growth and health of plants. An outstanding feature of the rhizosphere is that rhizodeposition and root turnover account for up to 40% of the carbon input into soil and clearly is the major driver for soil microbiological processes (Jones *et al*., 2009). Furthermore, roots interact with diverse populations of soil microorganisms which have significant implication for growth and nutrition (Mukerji *et al*., 2006; Brimecombe *et al*., 2007), either directly by influencing nutrient availability and uptake, or indirectly through plant (root) growth promotion. Soil nutrients are transferred towards the root surface through the rhizosphere or, in the case of roots associated with mycorrhizal fungi, through the mycorrhizosphere, prior to acquisition. It has become increasingly evident that root interactions with soil microorganisms are intricate and involve highly complex communities that function in very heterogeneous environments (Giri *et al*., 2005).

The genus *Nepenthes* belonging to the family Nepenthaceae is an insectivorous plant comprising of 86 species distributed in different areas of the world (Jebb and Cheek, 1977). *N. khasiana* is the only species found in India and it is restricted to a few areas of Meghalaya, situated in northeast India. It is a climbing under-shrub which ranges from a few centimeters to several meters in height. The presence of the pitchers renders ornamental importance to this plant and it is also reported to have ethno-medicinal

importance (Jain, 1987; Jeeva *et al*., 2007). The population of *N. khasiana* has dwindled in the last few decades due to deforestation, forest fires and excessive collection for trade and due to limestone and coal mining. It is now threatened in its natural habitat and is listed as an endangered plant in Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (Bordoloi, 1977). Therefore, the study of physico-chemical and biological properties of its rhizosphere soil is important to understand its growth in natural habitat for its *in situ* and *ex-situ* conservation.

Description of Study site

The natural population of the plant *N. khasiana* Hook. f.. in and around coal mining areas of Khliehriat, East Jaintia Hills District, Meghalaya, India, was selected for the present study. It is located between 25^0 21'25 N Latitude and 92^0 20'53 E Longitude. The climate is monsoonic where the rainy season occurs during mid-May to September and about 80% of the total annual rainfall occurs during this period. October and November is the transition period (autumn) between rainy and winter seasons. The period between December and February is characterized by cold and dry weather conditions.

Materials and Methods

Soil sampling

Soil samples were collected for a period of four months during winter and pre-monsoon season i.e. January to April 2013. The soil adhering to the roots of the plant was aseptically collected in a polythene bag. Care was taken to avoid contamination by other horizons. Each set of three samples was mixed thoroughly in a sterile polythene bag to make a composite soil sample. This was done to minimize local variation in the microbial population. The samples were brought to the laboratory and all aseptic precautions were taken to avoid contaminations.

Determination of soil Physico-chemical characteristics

Soil temperature was recorded at the time of sampling. The moisture content was determined by oven dry basis by drying a known amount of freshly collected soil sample in a hot air oven at 105°C for 24hours. Soil pH was read using electronic digital pH meter. Soil Organic Carbon and Total Nitrogen were determined by the method of Anderson and Ingram (1993), Available Phosphorous (P) by molybdenum blue method (Allen, 1974) and Exchangeable Potassium (K) by flame photometer (Jackson, 1972).

Isolation, identification and estimation of Fungi

Serial dilution plate method (Johnson and Curl, 1972) was followed for the isolation of fungi using Rose Bengal Agar medium (Martin, 1950). Three replicates were maintained for each sample. The inoculated Petri dishes were then incubated upside down at 25 ± 1˚C for 5 days in a BOD incubator. CFU of fungi per gram dry soil was determined on the dry weight basis. The fungal species were identified on the basis of their morphology and reproductive structures by consulting monographs by Subramaniam (1971), Barnett and Hunter (1972), Ellis (1971) and Domsch *et al.* (1980). The relationships between CFU and soil physico-chemical properties were analyzed by calculating Pearson's correlation coefficient (*r*) values. Shannon (1948) species diversity index and Simpson (1949) dominance index were calculated.

Results and Discussions

The soil temperature increases from January to April i.e. a minimum soil temperature of 8˚C was recorded in January and maximum of 19 ˚C was recorded in April. The ambient temperature was minimum in January and maximum in March. Both pH and soil moisture content were gradually increasing from January to April. Minimum pH value of 4.5 was recorded in January and a maximum value of 5.6 was recorded in April. Lowest soil organic carbon (%) of 1.8 was recorded during January and the highest value of 2.0 was recorded in March. Available phosphorous and exchangeable potassium was high during March while total nitrogen (%) did not show any variation during the study period (Fig. 1 & 2).

Highest fungal CFU of $25x10^3g^{-1}$ dry weight was observed in March and lowest fungal CFU of $11.9x10^3g^{-1}$ dry weight during January (Fig. 3). The increased in soil temperature and moisture content favours the growth of fungal population. A total number of 26 fungal species were isolated of which 2 species belonged to Oomycota, 6 belonged to Zygomycota, 17 belonged to Ascomycota and 1 sterile mycelia (Table1). *Cladosporium sphaerospernum*, *Penicillium janthinellum*, *P. brevicompactum* and *P. frequentans* were the most abundant species. *Penicillium* represented by 10 species was found to be the most common and dominant genera. Similar findings was reported by Gopal and Kurien (2013) where they found that *Penicillium* sp. was one of the

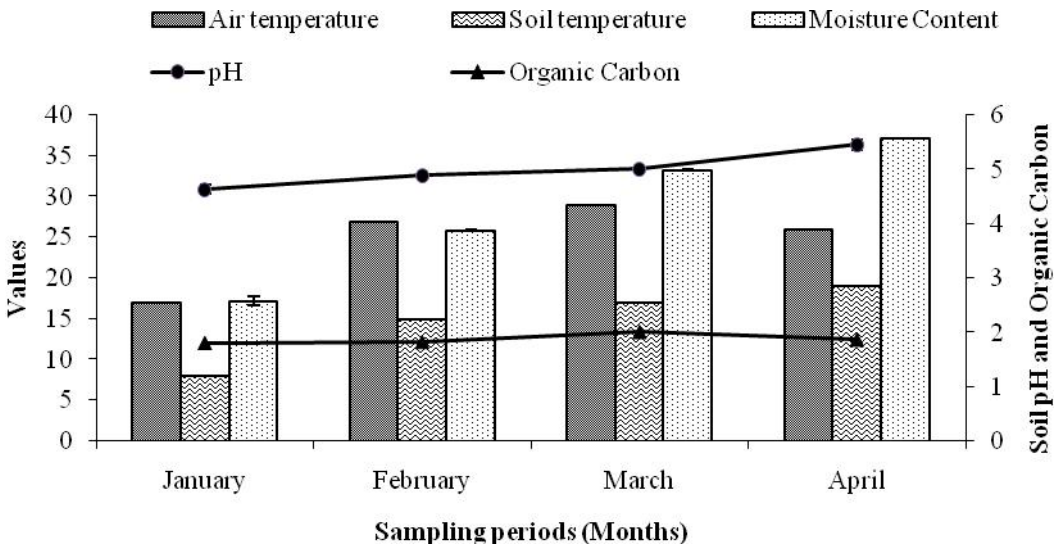

Fig. 1. Monthly variation of Air Temperature (˚C), soil temperature (˚C), moisture content (%), pH and organic carbon (%) of rhizosphere soil of *Nepenthes khasiana* Hook. f.

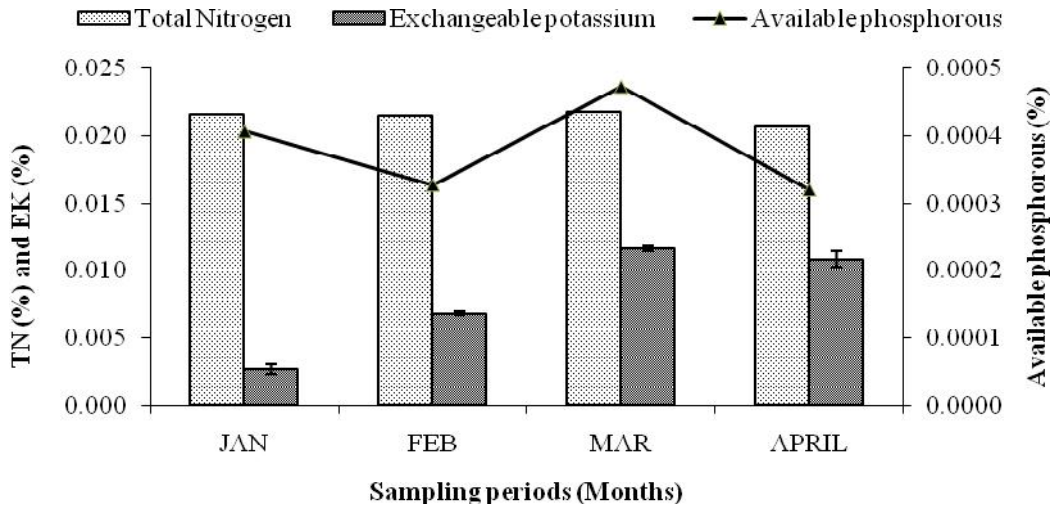

Fig.2. Monthly variation of total nitrogen (TN), exchangeable potassium (EK) and available phosphorous of rhizosphere soil of *Nepenthes khasiana* Hook. f.

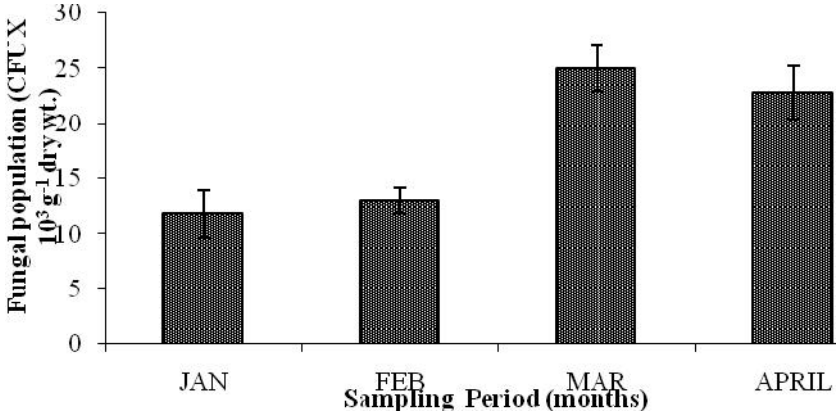

Fig. 3. Fungal population (CFU X 10^3 g^{-1} dry wt.) of rhizosphere soil of *Nepenthes khasiana* Hook. f.

Table. 1. Relative abundance of fungal species isolated from rhizosphere soil of *Nepenthes khasiana* Hook. f.

Sl. No.	Fungal species	Sampling Period			
		January	February	March	April
Oomycota- 2 Genera and 2 species					
1	*Pythium irregulare*	-	6.25	-	-
2	*Phytophthora cactorum*	16.67	-	-	-
Zygomycota- 5 Genera and 6 species					
3	*Mortierella humilis*	-	-	4.08	-
4	*M. parvispora*	-	-	-	12.5
5	*Mucor racemosus*	-	-	4.08	-
6	*Rhizomucor pusillus*	16.67	-	-	-
7	*Rhizopus oryzae*	-	6.25	-	-
8	*Zygorhynchus moelleri*	-	-	-	6.25
Ascomycota- 7 Genera and 17 species					
9	*Ceratocystis fimbriata*	-	6.25	-	-
10	*Cladosporium cladosporioides*	-	18.75	4.08	-
11	*C. sphaerospernum*	-	-	-	31.25
12	*Coccibioides immitis*	-	-	-	6.25
13	*Humicola grisea*	-	6.25	-	-
14	*Paecilomyces carneus*	-	12.5	-	6.25
15	*Penicillium atrovenetum*	-	-	-	6.25
16	*P. brevicompactum*	33.33	-	-	6.25
17	*P. canescens*	-	18.75	12.24	-
18	*P. chrysogenum*	-	6.25	-	-
19	*P. fellutanum*	-	6.25	-	-
20	*P. frequentans*	-	-	22.45	-
21	*P. janthinellum*	-	6.25	53.06	-
22	*P. lanosum*	16.67	-	-	-
23	*P. rubrum*	16.67	-	-	-
24	*P. sacculum*	-	-	-	6.25
25	*Talaromyces emersonii*	-	6.25	-	-
Sterile Mycelia-1					
26	White sterile mycelia	-	-	-	18.75

predominant fungi in rhizosphere soil of homestead crops, even though other fungi were also recorded. The high sporulation capacity and also antibiotics produced by *Penicillium* sp. may have prevented the growth of other fungal species. The plant species also equally influence the population and species composition of soil fungi (Hackl *et al*., 2000).

Shannon diversity index was highest in February and lowest in March whereas, Simpson dominance index was highest in March and lowest in February (Fig. 4). The species richness of fungal community is influenced by the selective determination ability of the host root exudates. The root exudates are known to play a great role in the determination of fungal communities in rhizosphere and rhizoplane regions (Broeckling *et al*., 2008). Neumann and Romhild (2001) were of the opinion that the shift in fungal community pattern in soil maybe due to root morphology and exudation pattern during plant development. Host plants also contribute to the seasonal variation in fungal isolates and their frequency of colonization (Gao *et al*., 2005).

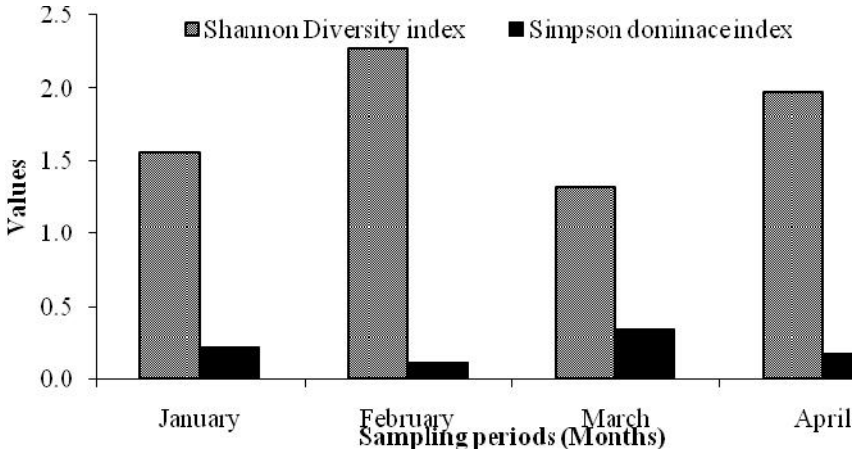

Fig.4. Shannon diversity and Simpson dominance index of fungi in rhizosphere soil of *Nepenthes khasiana* Hook. f.

CFU of fungi was positively correlated with soil temperature (r = 0.79, p≤0.05 and p≤ 0.01), moisture content (r = 0.89, p≤0.05, p≤0.01 and p≤0.001), pH (r = 0.73, p≤0.05 and p≤ 0.01), organic carbon (r = 0.86, p≤0.05, p≤0.01 and p≤0.001) and exchangeable potassium (r = 0.94, p≤0.05, p≤0.01 and p≤0.001) (Table 2). Temperature is one of the abiotic factors which influenced microbes in the rhizosphere soil which is evident by the positive correlation of fungal CFU with soil temperature. The development of fungal communities is also affected by temperature (Leung, 1998). In medium acid soils (pH 4.46 – 5.30), fungal abundance is higher in comparison with neutral soils (pH 6.68 – 7.90), since fungi prefer more acidic environments that bacteria (Grantina *et al*., 2011). There is a close relationship between microbial population and their activities and various edaphic factors, e.g, pH, soil organic matter, soil moisture and soil temperature regimes (Drouillon *et al*., 2005). The N, P, and K content are higher in and after raining season and organic content of natural soil also increases (Saravanakumar and Kaviyarasan, 2010). The soil pH, moisture content, Organic carbon, total nitrogen concentration and available K have positive correlations with fungal population (Shekh and Mohrir, 2012).

Table. 2. Correlation coefficient (r) values among fungal population with various physico-chemical characteristics in rhizosphere soil of *Nepenthes khasiana* Hook. f.

	ST	MC	pH	OC	TN	AP	EK
CFU	0.79 a,b	0.89 a,b,c	0.73a,b	0.86 a,b,c	NS	NS	0.94 a,b,c
ST		0.97	0.89	0.55	-0.50	NS	0.94
MC			0.93	0.61	-0.54	NS	0.97
pH				NS	-0.81	NS	0.80
OC					NS	0.69	0.79
TN						0.74	NS
AP							NS
EK							

Note: CFU = Colony Forming Unit, ST = Soil Temperature, MC = Moisture Content, OC = Organic Carbon, TN= Total Nitrogen, AP = Available Phosphorus and EK = Exchangeable Potassium. a = p≤0.05, b = p≤0.01 and c = p≤0.001

These observations suggest that the rhizosphere of *N. khasiana* harbours rich fungal diversity which varies temporally and hence, there is scope for isolation of large number of distinct groups of fungi which may play an important role in its growth and development.

Acknowledgments

The authors are grateful to the Head, Department of Botany, North-Eastern Hill University, Shillong and to Coordinator, CAS, Department of Botany, NEHU, Shillong for providing laboratory facilities and other infrastructures to carry out the work. The third author is also thankful to the University Grants Commission, Govt. of India for the fellowship.

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