ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 1, pp: (68-77), Month: January - March 2018, Available at: www.researchpublish.com

# Fungal Diversity of Rhizospheric and Non-Rhizospheric Soil of *Zingiber Officinale* Rosc. In West Khasi Hills District of Meghalaya, India

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***Abstract:*** **The present study was conducted to investigate the soil fungal diversity of *Zingiber officinale* Rosc. in Mawjiej, West Khasi Hills district of Meghalaya. Rhizospheric and non rhizospheric soil of *Z. officinale* was collected aseptically at monthly interval for a period of nine months i.e. April to December 2015. Altogether, 89 fungal species belonging to 35 different genera were isolated from the rhizospheric and non rhizospheric soil of *Z. officinale*. Maximum fungal Colony Forming Unit (CFU) was observed in June and minimum in December from both rhizospheric and non rhizospheric soil. *Gliocladium roseum* and *Paecilomyces lilacinus* were dominant in rhizospheric soil, whereas, species of *Penicillium* and *Fusarium oxysporum* were dominant in non rhizospheric soil in all the sampling periods. The investigation reveals that rhizospheric soil harbors more fungal population as compared to that of non- rhizospheric soil. In rhizospheric soil Fungal CFU showed significant positive correlation with moisture content (0.80, $p \leq 0.01$) and organic carbon (0.82, $p \leq 0.01$). Whereas, in non rhizospheric soil fungal CFU was found to have significant positive correlation with moisture content (0.76, $p \leq 0.05$), organic Carbon ($r = 0.89$, $p \leq 0.01$) and exchangeable potassium (0.69, $p \leq 0.05$).**

***Keywords:*** **Fungal diversity, Rhizospheric, Non-Rhizospheric, *Zingiber officinale* Rosc., Physicochemical properties.**

## 1. INTRODUCTION

Many studies have revealed that fungi are an important component of the soil microbiota constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions [1]. It was estimated 1.5 million fungal species are present in natural ecosystems, but only 5-10% has been described formally [2].The actual number of fungi is still unknown; however, only 5-13 % of the total estimated global fungal species have been described [3]. Research on fungal diversity provides a basis for estimating the functional role of fungi in ecosystems. The unique physico-chemical and biological characteristics of the soil associated with roots, compared to the soils away from the root and root surface are responsible for the enhanced microbial diversity together with increased number and activity of microorganisms in the rhizosphere [4]. Therefore, rhizosphere has been stated as a hotspot of microbial activities [5]. Despite their crucial role in the transport, storage, release and recycling of nutrients and also in the development and health of a plant [6] and their extraordinary impacts on ecosystems, relatively little is known about them.

*Zingiber officinale* Rosc. is an important commercial crop grown for its aromatic rhizomes [7] which are used as a spice and medicine [8]. India is one of the leading producer and exporter of ginger in the world. It is an important crop that earns a sizeable amount of foreign exchange for India [9]. The aims of the present study were to investigate the fungal diversity of the rhizospheric and non rhizospheric soil of *Z. officinale* Rosc. and also to determine its relationship with the various physico-chemical properties of the soil.

## 2. MATERIALS AND METHODS

**STUDY SITE:**

The study was conducted at Mawjiej village which is located at a geographical location of $25^0$47.272’ N and $91^0$04.644’ E, West Khasi Hills District of Meghalaya, India.

**SOIL SAMPLING:**

Soil sampling was done at a monthly interval for a period of one year (2015) for the crop growing season (April – December 2015). For the purpose of rhizospheric soil sampling, the growing rhizome was uprooted and the soil adhering to the rhizome was carefully collected in sterilized polythene bag whereas non rhizospheric soil was collected from the area surrounding the rhizome. The soil samples were stored at 4°C until further analysis.

**ISOLATION AND ENUMERATION OF FUNGAL POPULATION:**

Serial dilution plate method [10] was followed for the isolation of fungi using Rose Bengal Agar medium [11]. Three replicates were maintained for each sample. The inoculated Petri dishes were then incubated upside down at 25±1ºC for 5 days in a BOD incubator. The number of fungal colonies was counted and the population per gram dry soil was calculated by taking into consideration of the moisture content and dilution factor.

**IDENTIFICATION OF FUNGAL SPECIES:**

The fungal species were identified on the basis of their morphology and reproductive structures by consulting standard monographs. [12][13][14] [15].

**SOIL PHYSICO- CHEMICAL PROPERTIES:**

Soil temperature was noted at the time of sampling using soil thermometer. Soil pH was read by using electronic digital pH meter. The moisture content of the soil sample was determined by oven dried basis by drying 10 gram of soil in a hot air oven at 105°C for 24 hours and the dry weight was taken. Organic carbon, total nitrogen , available phosphorus, and exchangeable potassium was estimated by colorimetric method [16], micro Kjeldahl distillation and titration method [17], molybdenum blue method[18], and the ammonium acetate flame photometry method [17] respectively.

**DIVERSITY ANALYSIS:**

The diversity indices for the isolated fungal species were estimated following the methods of Shannon [19], and Simpson [20], and community similarity was determined using the methods of Sorenson [21].

**Shannon Diversity Index**

Shannon Index (H) = $-\Sigma$ pi ln pi

**Simpson Dominance Index**

Simpson Index (D) = $\Sigma pi2$

pi = n/N

Where n= number of individual species

N=Total number of individuals

Ln= Natural Log

**Sorenson’s Coefficient (CC) = 2C/ (S1+S2)**

Where, C= number of species the two communities have in common,

S1= Total number of species found in community 1

S2= Total number of species found in community 2

## 3. RESULTS

**Fungal population:**

Maximum fungal population was observed in June and minimum in December in both rhizospheric and non rhizospheric soil. The maximum rhizosphere effect was observed in November (2.08) and minimum in April (1.28) (Fig. 1).

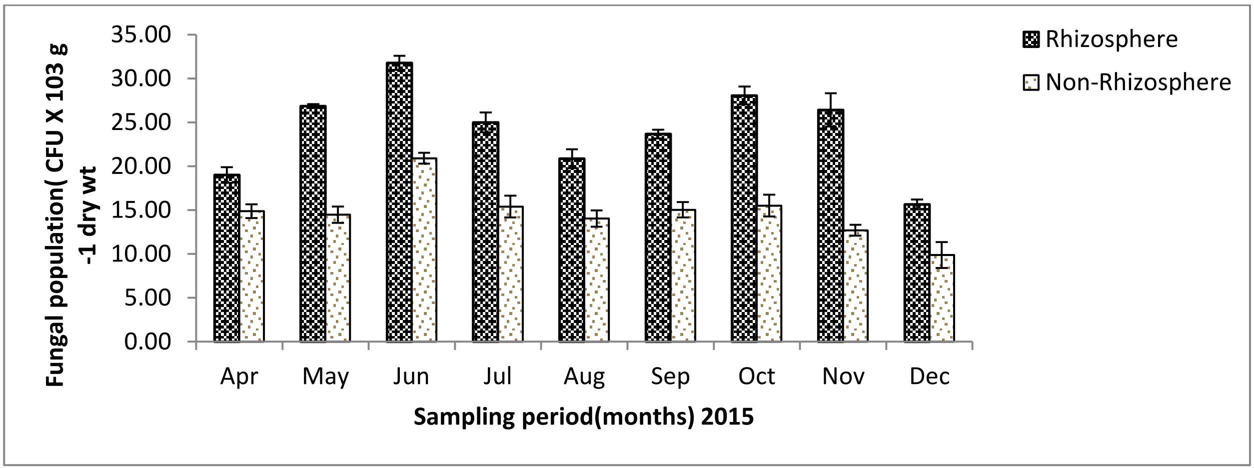


**Fig 1: Monthly variations of Colony Forming Units (CFUs) of fungi of rhizospheric and non rhizospheric soil of *Z.officinale* Rosc.**

Table 1 shows the list of fungal species isolated from both rhizospheric and non rhizospheric soil of *Z. officinale* Rosc. Altogether a total of 89 fungal species was isolated from both rhizospheric and non rhizospheric soil of which 3 species belonged to Oomycota, 14 species belonged to Zygomycota, 71 species to Ascomycota and 1 species to Basidiomycota and 2 sterile mycelia. A total of 71fungal species was isolated from the rhizospheric soil and 49 fungal species was isolated from the non -rhizospheric soil. 27fungal species were found to be common in both the soil samples. Thirty five genera were identified and the most dominant were *Penicillium* (19 Species), *Mortierella* (5species), *Acremonium* (5 species), *Paecilomyces* (6 species) and *Trichoderma* (5 species). *Gliocladium roseum* and *Paecilomyces lilacinus* were found to be the most dominant species isolated from the rhizospheric soil. Whereas in non rhizospheric soil *Penicillium canescens, P. chrysogenum, P. oxalicum, P. paradoxum, P. simplicissimum* and *Fusarium oxysporum* were found to be the most dominant species occurring in all the sampling period.

**Table 1: List of Fungal species Isolated from rhizospheric and non rhizospheric soil of *Zingiber Officinale* Rosc**

| Sl. No | Fungal species | Rhizospheric soil | Non-rhizospheric soil |
|---|---|---|---|
| **Phylum: Oomycota(2 genera, 3species)** | | | |
| **Class : Oomycetes** | | | |
| Order:Peronosporales | | | |
| 1. | *Phytophthora sp.* | + | + |
| Order: Pythiales | | | |
| 2. | *Pythium irregulare* | + | - |
| 3. | *Pythium sp.* | + | - |
| **Phylum: Zygomycota (5 genera , 14 species)** | | | |
| Order :Mucorales | | | |
| 1 | *Absidia cylindrospora* | - | + |
| 2 | *A.glauca* | + | + |
| 3 | *Gongronella butleri* | + | - |
| 4 | *Mucor circinelloides* | + | - |
| 5 | *M. hiemalis f. hiemalis* | + | - |
| 6 | *M. hiemalis f. silvaticus* | - | + |
| 7 | *Rhizopus nigricans* | - | + |
| 8 | *R. oryzae* | - | + |
| 9 | *R. stolonifer* | + | - |

| | | | |
|---|---|---|---|
| Order :Mortierellales | | | |
| 1 | *Mortierella alpina* | - | + |
| 2 | *M. minutissima* | + | - |
| 3 | *M. parvispora* | + | + |
| 4 | *M. ramanniana* var. *ramanniana* | + | + |
| 5 | *M. vinaceae* | + | - |
| **Phylum: Ascomycota (27 genera, 71 species)** | | | |
| **Class:Eurotiomycetes** | | | |
| | Order: Eurotiales | | |
| 1 | *Aspergillus fumigatus* | + | - |
| 2 | *A. flavus* | + | - |
| 3 | *Eupenicillium brefeldianum* | - | + |
| 4 | *E. javanicum* | + | - |
| 5 | *Paecilomyces carneus* | + | - |
| 6 | *P. farinosus* | + | - |
| 7 | *P. fumosoroseus* | + | - |
| 8 | *P. lilacinus* | + | + |
| 9 | *P. marquandii* | + | - |
| 10 | *P. variotii* | - | + |
| 11 | *Penicillium canescens* | + | + |
| 12 | *P. citrinum* | + | + |
| 13 | *P. chrysogenum* | + | + |
| 14 | *P. daleae* | + | + |
| 15 | *P. expansum* | + | + |
| 16 | *P. fellutanum* | + | + |
| 17 | *P. frequentans* | + | + |
| 18 | *P. janthinellum* | + | + |
| 19 | *P. jensenii* | + | - |
| 20 | *P. lanosum* | + | - |
| 21 | *P. oxalicum* | - | + |
| 22 | *P. paradoxum* | + | + |
| 23 | *P. purpurogenum* | - | + |
| 24 | *P. rubrum* | + | + |
| 25 | *P. rugulosum* | + | + |
| 26 | *P. simplicissimum* | + | + |
| 27 | *P. stoloniferum* | + | + |
| 28 | *Penicillium sp.* | + | - |
| 29 | *P. variabile* | - | + |
| | Order: Chaetothyriales | | |
| 30 | *Exophiala jeanselmii* | + | + |
| | Order: Onygenales | | |
| 31 | *Nannizzia grubyia* | + | - |
| 32 | *N. gypsea* | + | - |
| **Class : Sordariomycetes** | | | |
| | Order: Hypocreales | | |
| 33 | *Acremonium murorum* | + | - |
| 34 | *A. cerealis* | + | - |
| 35 | *A. fusidioides* | + | - |
| 36 | *A. kiliense* | + | - |
| 37 | *A. strictum* | + | - |
| 38 | *Acremoniella sarcinella* | - | + |
| 39 | *Baeuveria bassiana* | + | + |
| 40 | *B. brongniartii* | + | - |
| 41 | *Fusarium oxysporum* | + | + |
| 42 | *F. solani* | - | + |
| 43 | *F. sporotrichioides* | + | - |
| 44 | *Gliocladium catenulatum* | - | + |

| | | | |
|---|---|---|---|
| 45 | *G.roseum* | + | - |
| 46 | *Gliocladium sp* | + | - |
| 47 | *Myrothecium roridum* | + | - |
| 48 | *Nectria ventricosa* | + | + |
| 49 | *Scopulariopsis brumptii* | + | + |
| 50 | *Sesquicillium candelabrum* | + | - |
| 51 | *Trichoderma koningii* | + | - |
| 52 | *T. harzianum* | + | + |
| 53 | *T. pseudokoningii* | - | + |
| 54 | *T.polysporum* | + | - |
| 55 | *T. viride* | + | - |
| Order: Chaetosphaeriales | | | |
| 56 | *Gonytrichum macrocladum* | + | + |
| Order: Glomerellales | | | |
| 57 | *Verticillium alboatrum* | + | + |
| 58 | *V. chlamydosporum* | + | - |
| 59 | *V. dahliae* | + | + |
| 60 | *Verticillium* sp. | - | + |
| Order: Sordariales | | | |
| 61 | *Chaetomium sp.* | - | + |
| 62 | *Cladorrhinum sp.* | - | + |
| 63 | *Humicola fuscoatra* | + | - |
| 64 | *H.grisea* | + | + |
| **Class: Dothideomycetes** | | | |
| Order: Pleosporales | | | |
| 65 | *Curvularia lunata* | + | - |
| 66 | *Phoma eupyrena* | - | + |
| 67 | *Torula graminis* | - | + |
| Order: Capnodiales | | | |
| 68 | *Cladosporium cladosporioides* | + | - |
| 69 | *C. macrocarpum* | - | + |
| **Class: Ascomycetes** | | | |
| *Incertiordinis*(of uncertain order) | | | |
| 70 | *Oidiodendron griseum* | + | - |
| **Class: Leotiomycetes** | | | |
| Order: Helotiales | | | |
| 71 | *Scytalidium lignicola* | + | - |
| **Phylum:Basidiomycota( 1genera , 1 species)** | | | |
| **Class: Agaricomycetes** | | | |
| Order: Cantharellales | | | |
| 1 | *Rhizoctonia solani* | + | - |
| **STERILE MYCELIA** | | | |
| 1 | *White sterile mycelia* | + | - |
| 2 | *Yellow sterile mycelia* | + | + |

Monthly variations on Shannon diversity index is depicted in Fig. 2. Highest fungal diversity was observed in June and July in the rhizospheric and non rhizospheric soil respectively. While lowest diversity was found in December in both the rhizospheric and non rhizospheric soil. In most of the sampling months fungal diversity index was more in rhizospheric soil when compared to that of non rhizospheric soil. Simpson Dominance index was highest in December and lowest in August for rhizospheric soil, whereas in non-rhizospheric soil it was highest in December and lowest in July (Fig. 3). Sorenson's coefficient value was lowest in April and highest in November whereas there is no community similarity between rhizospheric and non rhizospheric soil recorded for the month of July (Fig. 4).

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International Journal of Life Sciences Research ISSN 2348-3148 (online)
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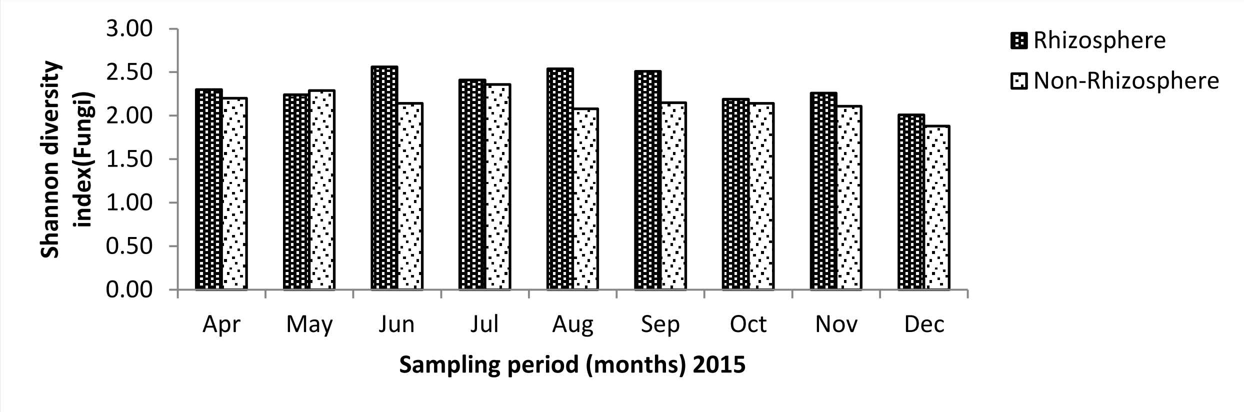


**Fig 2: Shannon diversity index of rhizospheric and non rhizospheric soil of *Z. officinale* Rosc**

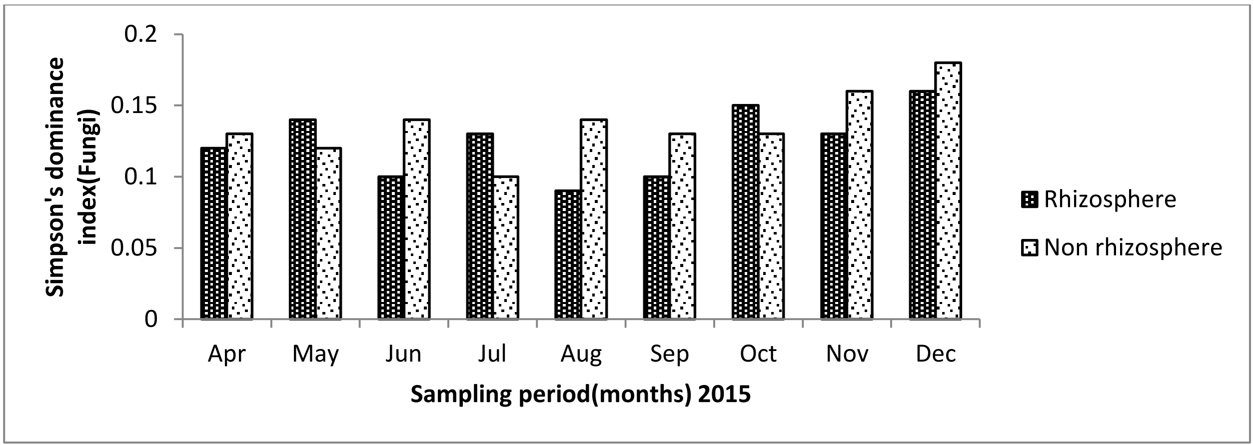


**Fig 3: Simpson Dominace index of rhizospheric and non rhizospheric soil of *Z. officinale* Rosc.**

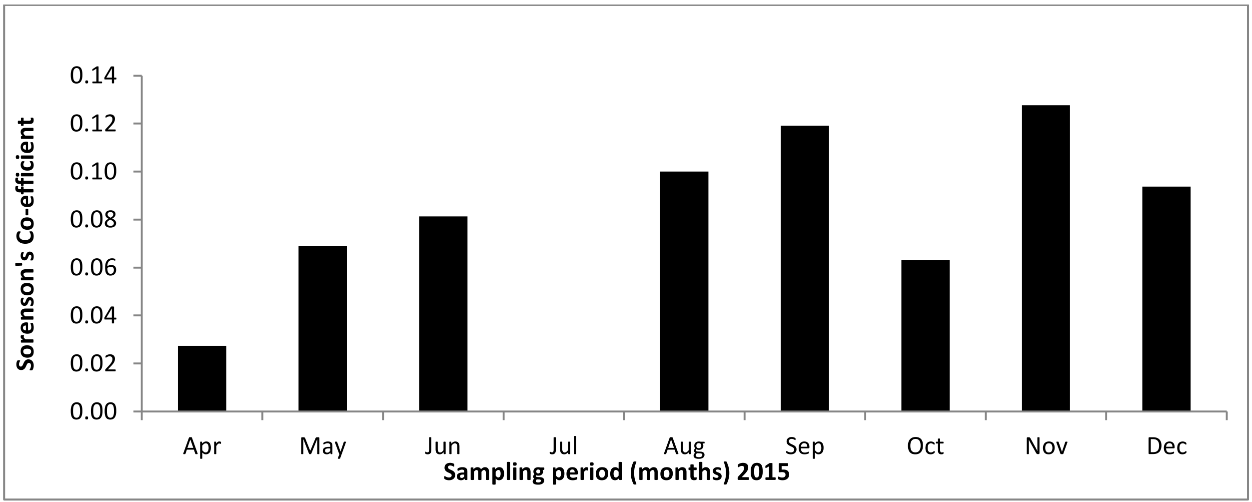


**Fig 4: Sorenson's Coefficient of rhizospheric and non rhizospheric soil of *Z. officinale* Rosc.**

## SOIL PHYSICO CHEMICAL ANALYSIS:

Soil temperature showed slight variation between rhizospheric and non rhizospheric soil. The rhizospheric soil pH was found to be slightly more acidic than non-rhizospheric soil. Soil moisture content, organic carbon and available phosphorus was relatively higher in rhizospheric soil than that of non rhizospheric soil in all the sampling period .Whereas variation in total nitrogen content and exchangeable potassium were observed in all the sampling period in both the soil samples. (Table 3) (Fig. 5 and Fig. 6).

**Table 2: Mean value of the various Physico-Chemical Properties of Rhizospheric and Non-Rhizospheric Soil of *Zingiber officinale* Rosc.**

| Soil properties | | Sampling period (months) 2015 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Apr** | **May** | **Jun** | **Jul** | **Aug** | **Sep** | **Oct** | **Nov** | **Dec** |
| **ST** | R | 27 | 26 | 25 | 30 | 31 | 26 | 24 | 25 | 23 |
| | NR | 28 | 28 | 26 | 37 | 31 | 27 | 30 | 28 | 27 |
| **pH** | R | 5.93 (±0.03) | 5.93 (± 0.03) | 5.43 (±0.03) | 5.26 (±0.01) | 5.75 (± 0.01) | 5.63 (± 0.09) | 5.78 (±0.01) | 5.74 (± 0.01) | 5.69 (± 0.01) |
| | NR | 6.07 (±0.09) | 5.83 (± 0.03) | 6.67 (±0.13) | 6.23 (± 0.03) | 6.50 (± 0.15) | 6.42 (± 0.01) | 5.98 (± 0.01) | 5.95 (± 0.01) | 5.96 (± 0.01) |
| **MC** | R | 21.37 (± 1.02) | 30.47 (± 0.68) | 29.80 ( ± 0.31) | 25.23 (± 0.32) | 23.13 (± 1.37) | 25.83 ( ± 0.13) | 26.97 ( ± 0.03) | 22.23 (± 0.13) | 20.83 (±0.09) |
| | NR | 26.03 ±1.13) | 26.27 ( ±0.79) | 26.70 ( ± 1.05) | 24.17 ( ± 1.28) | 23.93 ( ± 0.33) | 24.67 ( ± 0.24) | 24.07 ( ± 0.15) | 15.06 ( ± 0.23) | 12.13 ( ± 0.15) |
| **OC** | R | 1.30± (0.01) | 1.82 (±0.06) | 1.91 (±0.01) | 1.77 (±0.04) | 1.74 (±0.02) | 1.75 (±0.02) | 1.81 (±0.00) | 1.77 (±0.04) | 1.50 (±0.021) |
| | NR | 1.20 (±0.02) | 1.22 (±0.02) | 1.27 (±0.02) | 1.24 (±0.01) | 1.18 (±0.02) | 1.24 (±0.02) | 1.24 (±0.02) | 1.17 (±0.02) | 1.12 (±0.01) |
| **TN** | R | 0.289 (±0.02) | 0.275 (±0.01) | 0.261 (±0.00) | 0.256 (±0.00) | 0.266 (±0.01) | 0.177 (±0.03) | 0.238 (±0.00) | 0.238 (±0.06) | 0.284 (±0.01) |
| | NR | 0.275 (±0.02) | 0.298 (±0.01) | 0.256 (±0.00) | 0.233 (±0.01) | 0.219 (±0.02) | 0.233 (±0.02) | 0.242 (±0.00) | 0.252 (±0.00) | 0.252 (±0.00) |
| **AP)** | R | 0.0024 (±0.00) | 0.0019 (±0.00) | 0.0017 (±0.00) | 0.0016 (±0.00) | 0.0019 (±0.00) | 0.0021 (±0.00) | 0.0025 (±0.00) | 0.0025 (±0.00) | 0.0024 (±0.00) |
| | NR | 0.0016 (±0.00) | 0.0019 (±0.00) | 0.0012 (±0.000 | 0.0013 (±0.00) | 0.0016 (±0.00) | 0.0016 (±0.00) | 0.0017 (±0.00) | 0.0021 (±0.00) | 0.0018 (±0.00) |
| **EK** | R | 0.0025 (±0.00) | 0.0028 (±0.00) | 0.0128 (±0.00) | 0.0135 (±0.00) | 0.0124 (±0.00) | 0.0106 (±0.00) | 0.0113 (±0.00) | 0.0139 (±0.00) | 0.0188 (±0.00) |
| | NR | 0.0148 (±0.00) | 0.0166 (±0.00) | 0.0175 (±0.00) | 0.0166 (±0.00) | 0.0162 (±0.00) | 0.0127 (±0.00) | 0.0133 (±0.00) | 0.0145 (±0.00) | 0.0102 (±0.00) |

**Values are average of three replicates, standard error are given in parenthesis**

**Note: ST=Soil temperature, MC=Moisture content, OC = organic carbon, TN= total nitrogen, AP=available phosphorous and EK=exchangeable potassium ; R=Rhizosphere; NR=Non rhizosphere**

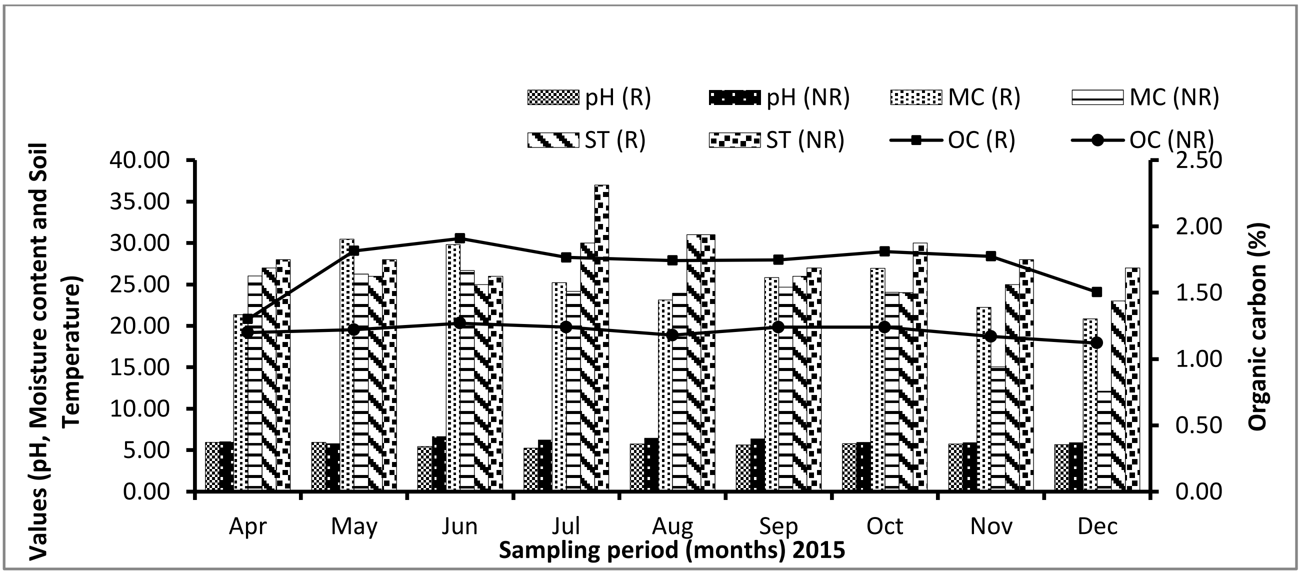


**Fig 5: Monthly variation of pH, moisture content (MC)(%) , Soil temperature(°C) and organic carbon(OC) (%) of rhizospheric and non-rhizospheric soil of *Z. officinale* Rosc.**

**Note: R="Rhizosphere", NR="Non-rhizosphere"**

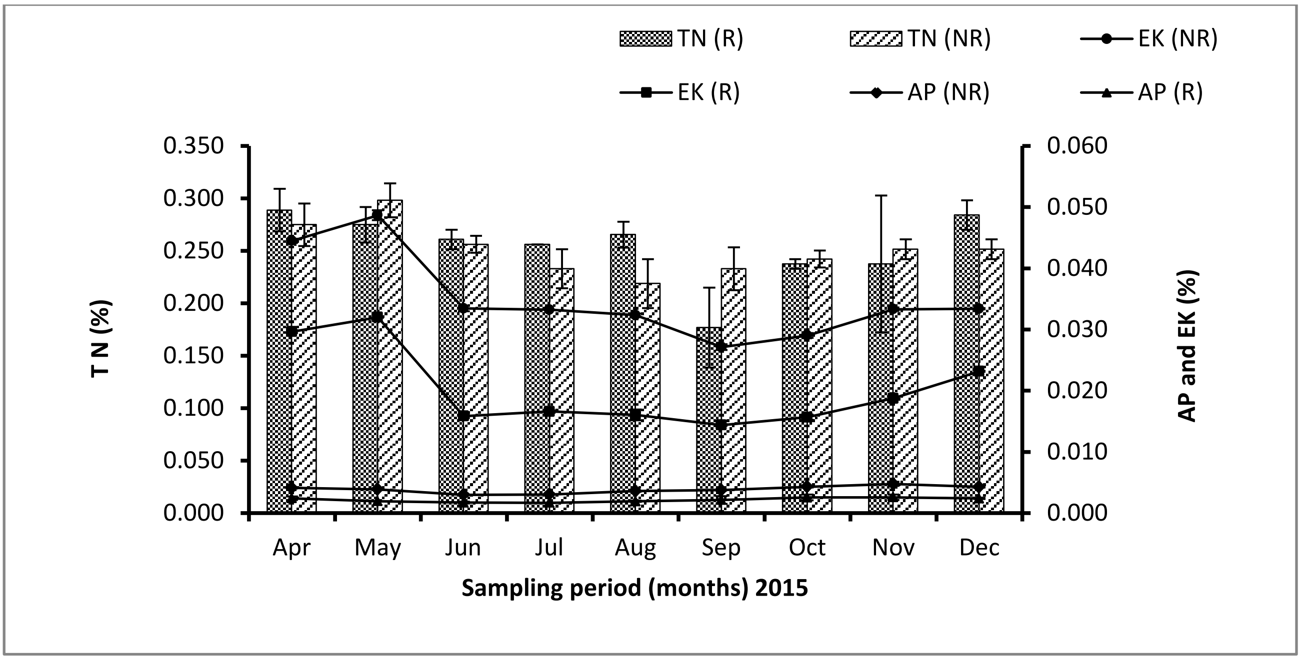


**Fig 6: Monthly variation of total nitrogen (TN), exchangeable potassium (EK) and available phosphorous (AP) of rhizospheric and non-rhizospheric soil of *Z. officinale* Rosc.**

**R="Rhizosphere",NR="Non-rhizosphere"**

**Table 4: Correlation coefficient (r) values among fungal population with variousphysico-chemical characteristics in rhizospheric and non rhizospheric soil of *Z. officinale* Rosc**

| | ST | pH | MC | OC | TN | AP | EK |
|---|---|---|---|---|---|---|---|
| **Rhizospheric soil** | | | | | | | |
| CFU | - | - | 0.80** | 0.82** | - | - | - |
| pH | - | - | - | - | - | - | - |
| MC | - | - | - | 0.74* | - | - | - |
| OC | - | - | - | - | - | - | - |
| TN | - | - | - | - | - | - | - |
| AP | - | - | - | - | - | - | - |
| EK | - | - | - | - | - | - | - |
| **Non-rhizospheric soil** | | | | | | | |
| | ST | pH | MC | OC | TN | AP | EK |
| CFU | - | - | 0.76* | 0.89** | - | -0.72* | 0.69* |
| pH | - | - | - | - | - | -0.75* | - |
| MC | - | - | - | 0.82** | - | - | 0.67* |
| OC | - | - | - | - | - | -0.67* | - |
| TN | - | - | - | - | - | - | - |
| AP | - | - | - | - | - | - | - |
| EK | - | - | - | - | - | - | - |

Values marked with * and ** are significant at $P \leq 0.05$ and $P \leq 0.01$ respectively; insignificant values are marked with '-'

Note: ST=Soil Temperature, MC=Moisture Content, OC =Organic Carbon, TN= Total Nitrogen, AP=Available Phosphorous and EK=Exchangeable Potassium.

**STATISTICAL ANALYSIS:**

In rhizospheric soil positive significant correlations was found between fungal population and moisture content ($r = 0.80$, $p \leq 0.01$) and organic carbon ($r = 0.82$, $p \leq 0.01$). Positive correlation is also observed between soil organic carbon and moisture content ($r = 0.74$, $p \leq 0.05$).

In non rhizospheric soil positive significant correlations was found between fungal population and moisture content (r = 0.76, p≤0.05), organic carbon (r = 0.89, p≤0.01) and exchangeable potassium (r = 0.69, p≤0.05). However, negative correlation was observed between CFU and available phosphorus (r = -0.72, p≤0.05). Among the various soil parameter positive correlation was recorded between soil moisture content and organic carbon(r = 0.82, p≤0.01) and exchangeable potassium (r = 0.67, p≤0.05). However, negative correlation was observed between pH and available phosphorus (r = -0.75, p≤0.05) and organic carbon and available phosphorus (r = -0.67, p≤0.05).

## 4. DISCUSSION

The investigation reveals that rhizospheric soil harbors more fungal population as compared to non-rhizospheric soil. Many workers have reported that greater numbers of microorganisms are present in the rhizospheric soil than in the non-rhizospheric soil.[22][23]

pH of rhizospheric soil was slightly more acidic as compared to the non- rhizospheric soil which can be attributed to the fact that respiration by plant roots and soil microorganisms released H+ ions .[24].

The CFU of fungi does not show any significant correlation with soil pH and similar findings has also been reported by Rousk *et al*., [25].

Significant positive correlation of fungal population with moisture content, organic carbon and exchangeable potassium indicates the influence of these parameters on the fungal count. Similar findings was also reported by Shekh *et al.*, (2012) [26] where the soil moisture content, Organic carbon, and available K have positive correlations with fungal population.

## 5. CONCLUSION

The present study proved that the rhizospheric soil has a higher fungal diversity in comparison to non -rhizospheric soil. It also indicates the important role of the various physico-chemical properties of the soil in influencing the fungal population.

## ACKNOWLEDGEMENT

The authors are grateful to the Head, Department of Botany, North-Eastern Hill University, Shillong, India, for providing laboratory facilities and other infrastructure to carry out the work. The financial support from the University Grant Commission, New Delhi in the form of National Fellowship for Higher Education for ST student (NFHE-ST) is gratefully acknowledged by the first author.

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