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Diversity of Culture Dependent Mycoflora of the Rhizosphere and Non Rhizosphere Soil of Maize (*Zea Mays* L.)

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Abstract The present investigation was carried out in pot experiment. Collection of rhizospheric and non-rhizospheric soil was done at fifteen days time intervals. For the isolation of the of culture dependent soil fungi, serial dilution plate method was followed using Rose Bengal Agar medium. Soil samples were collected aseptically from the rhizospheric and non-rhizospheric regions of maize from each experimental pot for a period of 105 days. Results revealed that fungal CFU (Colony Forming Unit) was higher in the rhizospheric soil than the non-rhizospheric soil throughout the sampling period. Altogether 41 fungal species were isolated from the rhizospheric and non-rhizospheric soil. Of which, 2 species belongs to Oomycota, 3 species to Zygomycota and 36 species to Ascomycota. A total number of 39 and 32 fungal species were isolated from rhizospheric and non rhizospheric soil respectively. Twenty eight fungal species were found to be common from both the soil samples. *Acremonium, Cladosporium* and *Penicillium* species were the dominant fungal species among the isolates. Shannon diversity index was high in rhizospheric soil community and Simpson dominance index was high in non rhizospheric soil community. Similarity Sorenson's Co-efficient index of the rhizospheric and non rhizospheric soil community was found to be highest during the 90th day of the sampling period.

Keywords *Colony Forming Unit; Fungi; Diversity Index; Dominance Index*

1. Introduction

Living plants create a unique habitat around the roots which is favourable for the proliferation and metabolism of numerous microorganisms. The microorganisms living in this complex region influences the health of a plant and also the surrounding soil ecosystems. Rhizosphere is directly influenced by root secretions and associated soil microorganisms. Much of the nutrient cycling and disease suppression needed by the plant occurs immediately adjacent to the roots.

Fungi are known to colonize diverse habitats and substrates and plays substantial role in plant health and productivity besides producing diseases. Studies on soil fungi have received much attention since the problems of soil mycological investigation was probed in by Adametz (1886). According to

Waksman (1952) the abundance of microorganisms in soil is influenced by various factors such as organic matter, soil reaction, moisture, temperature, aeration and nature of crop grown. The role of fungi in the soil is an extremely complex one and is fundamental to the soil ecosystem (Bridge and Spooner, 2001).

Fungi play a crucial role in the transport, storage, release and recycling of nutrients and also in the development and health of a plant (Thorn, 1997; Bridge and Spooner, 2001; Martin, 2001). Despite their extraordinary impacts on ecosystems, relatively little is known about them. Therefore, an improved knowledge of the structure and diversity of fungi can lead to a better understanding of their roles in soil ecosystems.

Both the generic compositions as well as size of the flora vary with the type of soil and with its physico-chemical characteristics. Among various physico-chemical characteristics of a soil, temperature, pH, moisture content, and organic carbon play important roles in regulating the population and activity of soil microbes.

The aim of the present investigation was to provide data on fungal population in the rhizospheric soil of maize and also to compare the rhizospheric fungal population with that of the non rhizospheric soil.

2. Materials and Methods

2.1. Collection of Soil Samples

Soil samples were collected from the rhizospheric and non-rhizospheric regions from each experimental pot at fifteen days intervals for a period of 105 days. For rhizospheric soil sampling, three maize plants were uprooted and complete root system with soil adhering to it was removed with the help of a sterilized digger and collected in sterilized polythene bags. For the non rhizospheric soil, samples were collected randomly from three experimental pots and mixed thoroughly to get a composite sample. The rhizospheric and non rhizospheric soil samples collected were stored at 4°C for further analysis.

2.2. Isolation and Enumeration of Fungi

Serial dilution plate method (Johnson and Curl, 1972) was followed for the isolation of rhizospheric and non rhizospheric fungi using Rose Bengal Agar medium (Martin, 1950). The soil particles closely adhering to the root system was collected aseptically by gently shaking the root system and was used for the isolation of rhizospheric fungi. For the non rhizospheric soil, samples were collected randomly from the three experimental pots and mixed thoroughly to get a composite sample.

Colony forming unit (CFU) of fungi was estimated by counting the number of fungal colonies. The CFU per gram soil was calculated on the dry weight basis.

2.3. Identification of Fungi

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 (1972) and Domsch et al. (1980).

2.4. Diversity Analysis

The diversity indices of the culturable fungi were estimated following the methods of Shannon (1948), and Simpson (1949), and community similarity was determined using the methods of Sorenson (1948).

Shannon Diversity Index

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

Simpson Dominance Index

Simpson Index (D) = Σp_i^2
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

2.5. Soil Physico Chemical Properties

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. Soil organic carbon was estimated by colorimetric method of Anderson and Ingram (1993).

2.6. Statistical Analysis

The relationship between the physico-chemical characteristics of the soil and the fungal count was determined by calculating the correlation coefficient (r) and each sample was analyzed in triplicates and averaged value was taken.

3. Results and Discussion

Fungal CFU exhibited variations throughout the sampling periods in both the rhizospheric as well as non-rhizospheric soils (Figure 1). Fungal CFU ranged from 9.2 to 36.9 x $10^3 g^{-1}$ dry soils for the rhizospheric soil and 2.8 to 24.8 x $10^3 g^{-1}$ dry soils for non- rhizosphere soil. Highest fungal CFU was observed on the 7th sampling period in rhizospheric soil and on the 6th sampling period in the non-rhizospheric soil (Table 1).

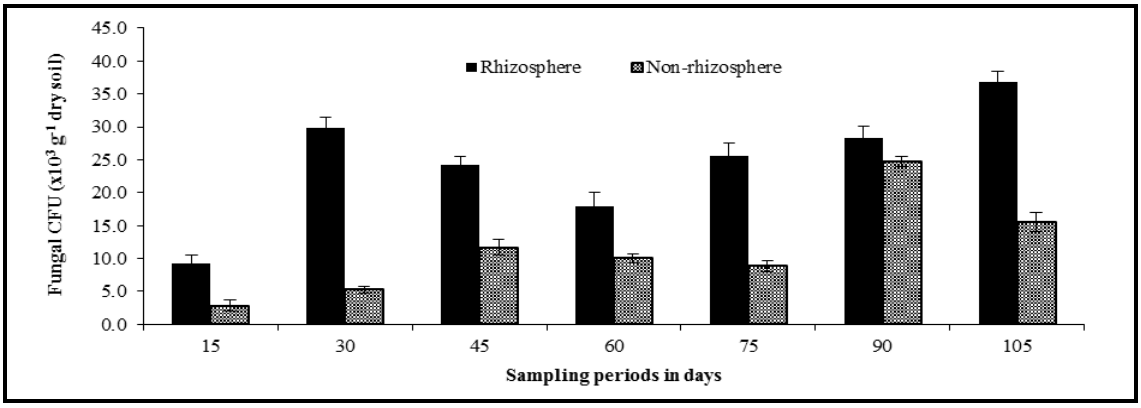

Figure 1: *Fungal CFU (x10^3 g^{-1} dry soil) of Rhizosphere and Non-rhizosphere soil of Maize (Zea mays L.)*

Table 1: *Fungal CFU (x10^3 g^{-1} dry soil) of Rhizosphere and Non- Rhizosphere soil of Maize (Zea mays L.)*

Sampling Periods (days)	Rhizosphere Soil	Non- Rhizosphere Soil
15	9.19±1.40	2.81±0.84
30	29.83±1.68	5.28±0.51
45	24.18±1.33	11.69±1.19
60	17.98±2.00	10.04±0.70
75	25.59±1.87	8.82±0.89
90	28.37±1.71	24.79±0.79
105	36.86±1.56	15.48±1.42

Higher fungal CFU in the rhizospheric soil than that of the non- rhizospheric soil may be due to the different types of substances released from the roots such as carbohydrate (sugars and oligosaccharides), organic acids, vitamins, nucleotides, flavonoids, enzymes, hormones, and volatile compounds (Prescott, et al., 1999) that may have stimulated the microbial activities in the root region as compared to the non rhizospheric soil. The exudate from the roots acts as a signal which stimulates the biological and physical interactions between roots and soil microorganisms (Nannipieri, et al., 2003). The exudate modifies the biochemical and physical properties of the rhizospheric soil and contributes to root growth and plant survival resulting in a dense and active microbial population in the root region.

Plants secrete many compounds through their roots to serve symbiotic functions in the rhizosphere. The release of organic compounds by the roots results in dramatic changes in the physical, biological and chemical nature of the soil and also sustains the continuum of microbial populations colonizing niches from the plant's interior and into the bulk soil which has an impact upon their environment. Plant roots exert strong effects on the rhizosphere by providing suitable ecological niches for microbial growth (Bais, et al*.,* 2006). The rhizosphere contains many bacteria that feed on sloughed-off plant cells, termed *rhizodeposition*, and the proteins and sugars released by roots and it is also known that maize seeds exude a large variety of compounds that affect and modify the surrounding soil (Vilchez, et al*.,* 2000). Furthermore, it is known that the total number of microbes is higher in the rhizosphere soil as compared to the bulk soil due to the continuous supply of nutrients via the root exudates (Kowalchuk et al., 2002 and Nunes da Rocha et al*.,* 2009).

Table 2 depicts the list of fungal species isolated from rhizospheric and non-rhizospheric soil of maize plant. Altogether 41 fungal species were isolated from the rhizosphere and non-rhizosphere soil. Of which, 2 species belonged to Oomycota, 3 species to Zygomycota and 36 species to Ascomycota. A total number of 39 and 32 fungal species were isolated from rhizosphere and non-rhizosphere soil respectively. Species of *Acremonium, Cladosporium* and *Penicillium* were the dominant fungal species among the isolates.

Table 2: *List of Fungal Species Isolated from Rhizosphere & Non-Rhizosphere Soil of Maize (Zea Mays L.)*

Sl. No.	Fungal species	Rhizosphere	Non-rhizosphere
	OOMYCOTA (2 genera, 2 species)		
1	*Phytophthora cactorum*	+	-
2	*Pythium irregulare*	+	+
	ZYGOMYCOTA (3 genera, 3 species)		
1	*Mortierella ramanniana*	+	-
2	*Mucor racemosus*	+	+
3	*Rhizopus oryzae*	+	-
	ASCOMYCOTA (19 genera, 36 species)		
1	*Acremonium cerealis*	-	+
2	*A. kiliense*	+	+
3	*A. strictum*	+	-

4	*Alternaria alternata*	+	+
5	*A. tenuissima*	+	+
6	*Arthroderma tuberculatum*	+	+
7	*Aspergillus fumigatus*	+	+
8	*Cladosporium cladosporioides*	+	+
9	*C. macrocarpum*	+	+
10	*C. sphaerospermum*	+	+
11	*Exophiala jeanselmei*	-	+
12	*Fusarium solani*	+	+
13	*Geotrichum candidum*	+	+
14	*Gliocladium catenulatum*	+	+
15	*Humicola fuscoatra*	+	+
16	*H. grisea*	+	+
17	*Mammaria echinobotryoides*	+	-
18	*Nannizzia grubyia*	+	+
19	*Nectria ventricosa*	+	-
20	*Paecilomyces carneus*	+	+
21	*Penicillium brevicompactum*	+	+
22	*P. canescens*	+	+
23	*P. daleae*	+	+
24	*P. fellutanum*	+	+
25	*P. janthinellum*	+	+
26	*P. jensenii*	+	+
27	*P. lanosum*	+	+
28	*P. restrictum*	+	-
29	*P. sacculum*	+	-
30	*P. simplicissimum*	+	-
31	*P. spinulosum*	+	+
32	*Phoma eupyrena*	+	+
33	*Scytalidium lignicola*	+	+
34	*Trichoderma harzianum*	+	-
35	*T. koningii*	+	+
36	*Verticillium dahliae*	+	-

Species such as *Phytophthora cactorum, Mortierella ramanniana, Rhizopus oryzae, Acremonium strictum, Mammaria echinobotryoides, Nectria ventricosa, Penicillium restrictum, P. sacculum, P. simplicissimum, Trichoderma harzianum* and *Verticillium dahlia* were restricted only to the rhizospheric soil. Whereas *Acremonium cerealis* and *Exophiala jeanselmei* were isolated only from the non-rhizospheric soil. Twenty eight fungal species were found to be common in both the soil samples.

Rhizospheric and non-rhizospheric soil exhibited similar fungal species as fungi residing in the rhizosphere most likely have originated from the surrounding bulk soil and might have thrived under conditions prevailing in the neighbourhood of plant roots. It must therefore, be assumed that fungal communities in the rhizosphere form a subset of the total fungal community present in bulk soils (Curl and Truelove, 1986).

In most of the sampling periods Shannon diversity index was higher in rhizospheric soil than that of the non rhizospheric soil (Figure 3), whereas, Simpson dominance index was higher in non-rhizosphere soil (Figure 4). Sorenson's coefficient value was lowest in 15th day of sampling period, whereas, it was highest during the 90th day of sampling period (Figure 6).

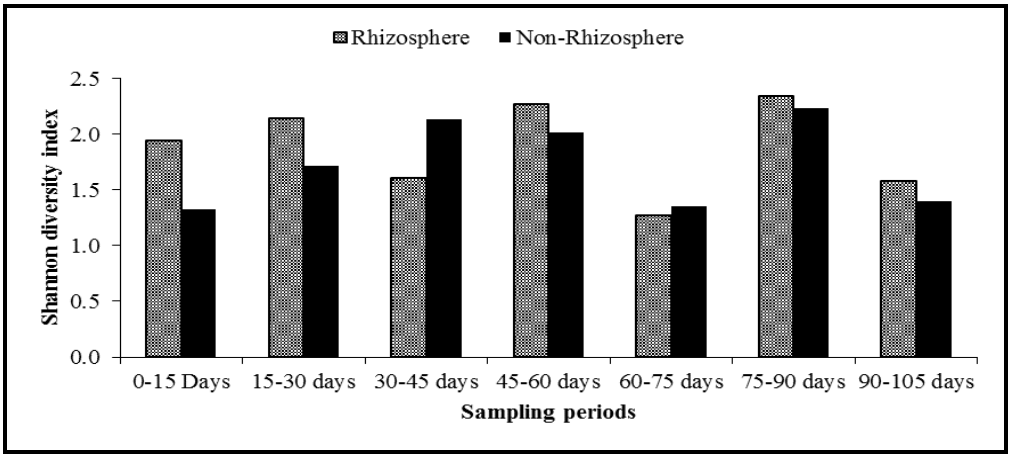

Figure 2: *Shannon Diversity Index of Rhizosphere and Non-Rhizosphere Soil of Maize (Zea mays L.)*

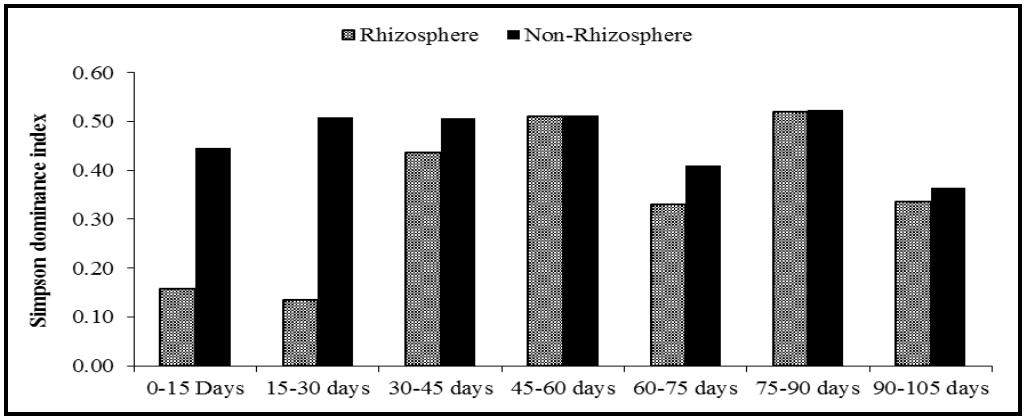

Figure 3: *Simpson Dominance Index of Rhizosphere and Non-Rhizosphere Soil of Maize (Zea mays L.)*

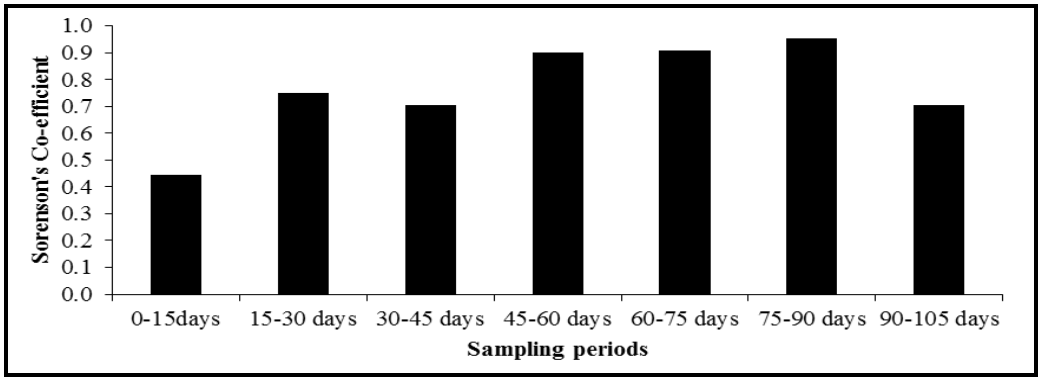

Figure 4: *Sorenson's Co-Efficient Index of Rhizosphere and Non-Rhizosphere Soil of Maize (Zea mays L.)*

3.1. Physico-Chemical Properties of Rhizosphere and Non-Rhizosphere Soil

Table 3 depicts the mean values of the physico-chemical properties of rhizospheric and non-rhizospheric soil of maize plant with standard errors (SE). pH of soil was acidic in both rhizospheric and non- rhizospheric soil (Figure 5). pH of soil ranged between 5.3 and 6.5 in rhizosphere soil and 5.6 and 6.2 in the non- rhizosphere soil. 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 (Sinha, et al., 2009). Also respiration leads to carbon dioxide (and

eventually to bicarbonate/carbonic acid) generation. In addition to respiration of the roots themselves, the rhizosphere is very rich in carbon due to the prokaryotes to fungi to small animals living and respiring in the rhizosphere more than in the bulk soil.

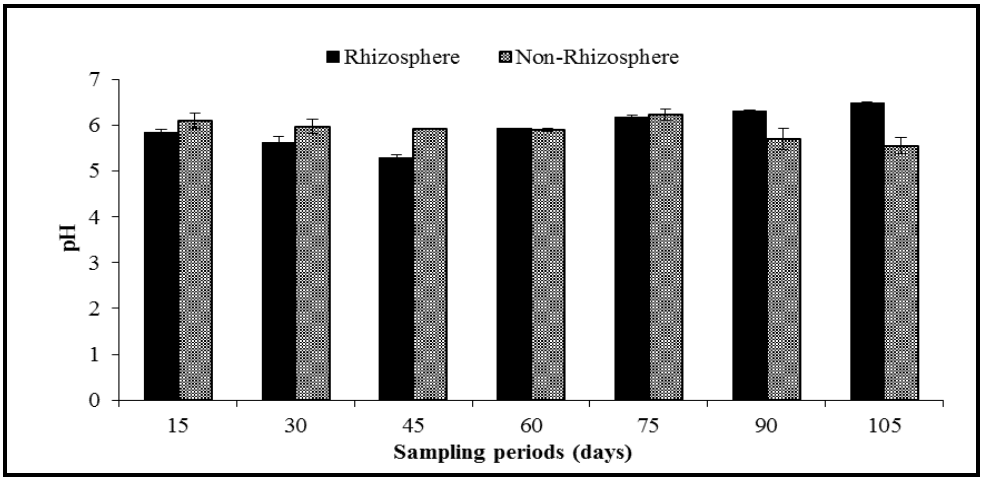

Figure 5: *pH of Rhizosphere and Non-Rhizosphere Soils of Maize (Zea mays L.)*

Table 3*: Mean values of physico-chemical properties of Rhizosphere and Non–Rhizosphere soil of Maize (Zea mays L.) with standard errors (SE)*

Soil properties		Sampling Period (days)						
		15	30	45	60	75	90	105
pH	R	5.84±0.06	5.62±0.13	5.28±0.09	5.93±0.01	6.17±0.05	6.32±0.00	6.49±0.02
	NR	6.10±0.17	5.97±0.15	5.92±0.01	5.89±0.03	6.23±0.13	5.70±0.23	5.55±0.18
MC	R	30.84±0.87	30.41±0.51	25.58±0.06	22.10±0.51	30.92±0.61	29.48±0.72	31.24±0.66
	NR	28.51±0.69	29.74±1.13	25.87±0.08	20.36±0.22	31.93±0.41	27.38±0.01	30.99±1.28
OC	R	0.81±0.01	0.88±0.02	1.45±0.10	1.08±0.05	0.41±0.05	0.37±0.05	0.50±0.04
	NR	0.74±0.02	0.91±0.02	1.11±0.03	0.11±0.01	0.24±0.03	0.51±0.04	0.34±0.01

Note: R*=Rhizosphere soil;* ***NR*** *=Non- Rhizosphere soil;* ***MC*** *= Moisture content (%);* ***OC*** *= Organic carbon (%)*

Throughout the sampling periods, the moisture content was found to be almost similar in rhizospheric as well as non- rhizospheric soil (Figure 6). This may be due to regular watering of the plant. Moisture threshold affects the availability of oxygen in some soils, if it's too high, microbial growth is restricted. Soil moisture is one of the key factors influencing soil microbial activity and soil organic matter decomposition (Brady and Weil, 2002). When soils become dry, it causes a decrease in enzyme activity (Sardans and Penuelas, 2005) and it reduces the thickness of water films on soil surfaces and therefore, the rate of diffusion of substrates to microbes (Stark and Firestone, 1995).

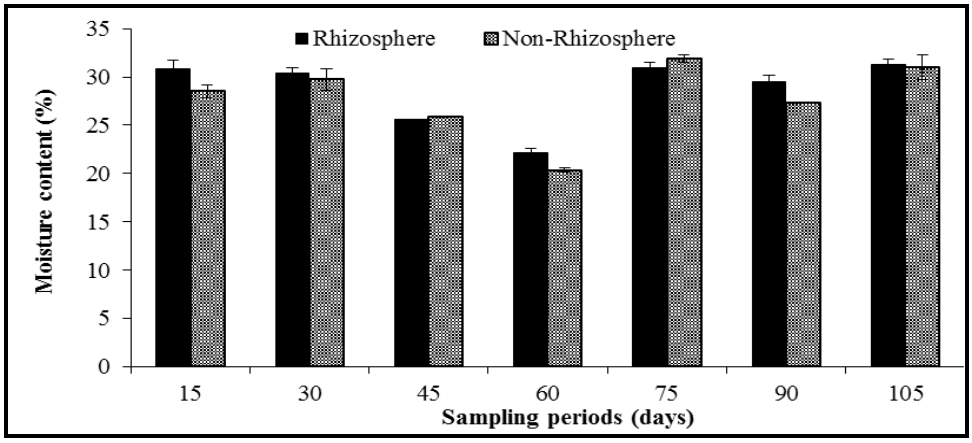

Figure 6: *Moisture content of Rhizosphere and Non- Rhizosphere soil of Maize (Zea mays L.)*

The organic carbon content in the rhizospheric soil was higher as compared to the non- rhizospheric soil (Figure 7). The soil organic carbon ranged from 0.37 to 1.49% in the rhizospheric soil and 0.11 to 1.11% in the non- rhizospheric soil. Increase in soil organic carbon in the rhizosphere is affected by rhizodeposition, which involves wide range of processes by which carbon enters the soil including root cap and border cell loss, death and lysis of root cells (cortex, root hairs etc), flow of carbon to root associated symbionts living in the soil, gaseous losses, and leakage of solutes from living cells. Apart from this, most plant in natural and semi natural vegetation systems forms symbiotic associations with fungi which facilitates the flow of carbon to and through this symbiotic interface resulting in increased carbon content in the root region compared to the bulk soil (Leake, et al., 2004).

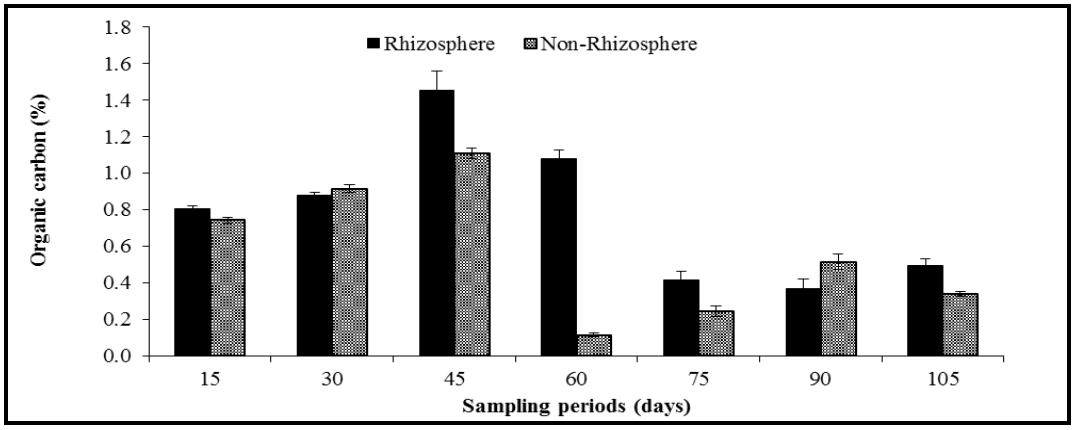

Figure 7: *Organic carbon of Rhizosphere and Non-Rhizosphere soil of Maize (Zea mays L.)*

3.2. Statistical Analysis

Table 4 depicts the correlation coefficient values between fungal CFU and the physico-chemical properties of rhizospheric and non- rhizospheric soil of maize plant.

In rhizospheric soil, organic carbon was found to have significantly negative correlation with moisture content ($r = -0.69$; $p \leq 0.05$) and pH ($r = -0.89$; $p \leq 0.05$ and $p \leq 0.01$). In non- rhizospheric soil, CFU of fungi was negatively correlated with pH ($r = -0.71$; $p \leq 0.05$).

Table 4: *Correlation coefficient (r) values of fungal CFU with physico-chemical properties of Rhizosphere and Non-Rhizosphere soil of Maize (Zea mays L.)*

Soil properties	MC	pH	OC
Fungal CFU	NS	NS	NS
MC		NS	-0.69
pH			-0.89
	Non-Rhizosphere Soil		
Fungal CFU	NS	-0.71	NS
MC		NS	NS
pH			NS

Note: MC= Moisture content; **OC**= Organic carbon; **NS** = Not significant
Insignificant values are marked with 'NS'

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