Int.J.Curr.Microbiol.App.Sci (2016) *5*(3): 363-372

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 3(2016) pp. 363-372**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.503.043

Seasonal Diversity of Rhizospheric Microfungi in Two Different Age Group of Tea Plantation in Tripura, Northeast India

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Keywords

Tea plantation, Soil microfungi, Seasonal diversity, Soil physiochemical properties.

Article Info

Accepted:
15 February 2016
Available Online:
10, March 2016

A B S T R A C T

Seasonal diversity of rhizospheric microfungi associated with two different age group of tea plantation was examined by using serial dilution and soil plate methods on the Czapek's Dox Agar medium supplemented with antibiotic Streptomycin. Identification and characterization of soil microfungi were done with the help of accessible manuals of fungi. Soil physico-chemical properties including soil pH, electrical conductivity, moisture content, water holding capacity, bulk density, soil texture, organic carbon, organic matter, nitrogen, potassium and phosphorus content were also analyzed. A total of 32 fungal species and white sterile hyphae were isolated and identified from both the selected plantation sites. Among the identified fungal species, 2 species are belonging to the genus *Alternaria*, *Fusarium* and *Rhizophus*, 6 species belong to *Aspergillus*, 4 species belong to the genus *Penicillium* while 3 species are belong to the genus *Verticillium*. Only one species from each of the genus *Bispora*, *Botrytis*, *Candida*, *Cephalosporium*, *Cladosporium*, *Culvularia*, *Dendropsis*, *Giocladium*, *Giotrichum*, *Helminthosporium*, *Humicola*, *Nigrospora and Trichoderma* were also isolated and identified during the course of the work. Sterile hyphae were highly isolated from both the plantation sites. *Aspergillus niger*, *Humicola brevis* and *Verticillium effusum* were isolated throughout the year. The dominant genera in both the selected plantation were *Aspergillus* and *Penicillium*.

Introduction

Soil is a most precious natural resource that contains most diverse assemblages of living organisms (Swer *et al.*, 2011) and the distribution of such organisms is influenced by the abundance and nature of the soil organic content, soil texture, surface vegetation as well as by other soil and climatic conditions (Waksman, 1944; Marschner *et al.*, 2003). Rhizosphere is considered as a hotspot of microbial activities (Brimecomb *et al.*, 2007). Fungi are fundamental for soil ecosystem

functioning (Warcup, 1951) as they contribute appreciably in the recycling of nutrients in natural and modified ecosystems (Gadd, 2004). It was estimated that 1.5 million fungal species are present in natural ecosystems, but only 5-10% has been described formally (Hawksworth, 2001). Soil and rhizospheric fungal diversity is much higher than it was formerly thought (Vandenkoornhuyse *et al*., 2002; Gams, 2007). Frequent studies on soil fungi has gradually grown up the perception that soil fungal flora may vary depends on its native soils (Shi *et al*., 2002; Gleason *et al*., 2004; Burges, 1939). The relative abundance of individual species has been considered as measures of functional activities of the group in the particular habitat (Kjøller and Struwe, 1982, 1987).

In this connection the present investigation was carried out to enumerate the rhizospheric microfungal diversity and the percentage of relative abundance along with certain soil physicochemical properties of the rhizospheric soil collected from two different age group of tea plantation of Tripura, northeast India.

Materials and Methods

Collection of the Soil Sample

The rhizospheric soil samples were regularly collected from Ganganagar tea estate where both the desired i.e., 5 and 20 year old plantations are found during the period January-December, 2013. The age of both the plantation were confirmed by discussing with the labour of the tea estate. The five year old plantation was considered as a young plantation where as twenty years old plantation is considered as mature plantation. At first the sampling area was cleaned by removing dry leaves and other unwanted materials. Samples were collected from 10 cm below the soil surface by digging it and measured by using proper centimeter scale. The soil samples were mixed well after the collection of the samples from the rhizosphere of the desired plantation. The soil sample were collected in sterilized polythene bags and brought into the laboratory for further analysis.

Soil Analysis

For pH and electrical conductivity, 10 g of soil was dissolved in 50 ml of distilled water and stirred for 20 min. This solution was kept overnight and then the soil pH and electrical conductivity was measured using a digital pH and conductivity meter respectively. Moisture content of the soil sample was determined by following the method of (Jackson, 1967). Soil texture was determined by Boyoucous hydrometric method given by Allen *et al*. (1974). Water holding capacity was determined following the method outlined by Allen *et al*. (1974). Bulk density of the soil sample was determined by (Anderson and Ingram, 1993) method. The Organic Carbon was determined by using Walkley-Black (1934) method.

The soil available Nitrogen was estimated following Black (1982) method. Available Phosphorus and Potassium of soil were determined using the method of Jackson (1978).The percentage of the organic matter present in the soil sample were determined by following the formula Percentage of organic matter=Percent of carbon×1.724.The factor 1 .724 is based on the assumption that the carbon is only 58%of organic matter.

Preparation of Media and Inoculation

The soil micro fungi were enumerated by Serial dilution (Waksman, 1927) and soil

plate method (Warcup, 1950). The culture media used for the isolation of the soil micro fungi was Czapek's Dox agar. The desired glass wares and the prepared media were sterilized in the autoclave and transferred to the laminar air flow. Then One percent antibiotic (Streptomycin) was added in all the petridishes for preventing them from the contamination of bacteria.

The media is then poured in the petri plates and allowed to cool at room temperature. After inoculation, the plates were kept for minimum incubation for 4-6 days at 25^0C until the colonies grow well in dust free chamber. Isolates which did not produce spores were treated as sterile mycelium (Lacap *et al*., 2003).

Lacto Phenol Cotton Blue Mounting

A portion of the mycelium of the representative colonies was picked up with the help of a pair of needles and grease free semi permanent slides were prepared using Lacto phenol cotton blue. The slide was gently heated in a spirit lamp to release the air bubbles, if any present inside the cover glass. The excess stain was removed using tissue paper and observed under microscope for the identification of the fungus.

Identification of Fungi

The fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys. The species was then identified by using the identification manual (Gilman, 1957; Barnett and Hunter, 1972).

Data Analysis

All the calculation was done in Microsoft Excel, 2007. Percentage of relative abundance of the fungal species was calculated using the following formula

$$\textbf{\% of relative abundance} = \frac{\text{Total no of colonies of a particular genus / species}}{\text{Total no of colonies of all the genera / species}} \times 100$$

Results and Discussion

Soil Physicochemical Properties

The pH of the rhizospheric soil sample of both the selected tea plantation sites was found to be acidic and soil of the mature plantation is slightly more acidic than the young plantation. Moisture content, Water holding capacity and bulk density was higher in 20 year tea plantation than 5 year old plantation. The clay content of both the soil sample of selected plantation is almost similar where as the slit content of the 5 year old plantation is higher than the 20 year old plantation. Sand content is high in 20 year old plantation than that of 5 year old plantation. Organic carbon, nitrogen, phosphorus, potassium and organic matter content are found to be higher in 20 year old plantation in comparison with the 5 year old tea plantation.

Rhizospheric Microfungi

In this present investigation, a total of 32 fungal species and white sterile hyphae were isolated and identified from both the selected plantation sites. Among the identified fungal species 2 species are belonging to the genus *Alternaria*, *Fusarium* and *Rhizophus*, 6 species belong to *Aspergillus*, 4 species belong to *Penicillium* while 3 species are belong to the genus *Verticillium*. Only one species of each of the genus *Bispora, Botrytis, Candida, Cephalosporium, Cladosporium, Culvularia, Dendropsis, Giocladium, Giotrichum, Helminthosporium, Humicola, Nigrospora*

and Trichoderma were also isolated and identified during the course of the work. Sterile hyphae were highly isolated from both the plantation sites. This present study revealed that *Aspergillus niger, Humicola brevis* and *Verticillium effusum* were isolated throughout the year. The dominant genera in both the selected plantation were *Aspergillus* and *Penicillium*.In winter, *Penicillium restrictum* has the highest percentage of relative abundance and *Aspergillus flavus* has the lowest percentage of relative abundance in 5 year old plantation where as *Rhizophus nigricans and Culvularia geniculatum* exhibited the highest and lowest percentage of relative abundance respectively in 20 year old tea plantation. In Pre monsoon *Aspergillus niger* has the highest percentage of relative abundance and *Alternaria tennius* has the lowest percentage of relative abundance in 5 year as well as 20 year old tea plantation. During monsoon *Verticillium effusum and Aspergillus niger* has the highest percentage of relative abundance where as *Culvularia geniculatum has* the lowest percentage of relative abundance in 5 year old plantaion. *Aspergillus niger and Giotrichum sp* have the highest and lowest percentage of relative abundance respectively in 20 year old plantation in monsoon. In Retreating Monsoon, *Aspergillus niger* has the highest percentage of relative abundance and *Aspergillus candidus and Verticillium effusum* has the lowest percentage of relative abundance in 5 year old plantation where as *Aspergillus niger* exhibited the highest percentage of relative abundance in 20 year old tea plantation.

Chaudhary and Sachar (1934), Trenser *et al*.(1954), Saksena (1955), Miller *et al*. (1957) and Saksena and Sarbhoy (1964),Rama rao (1969) and Persiani *et al*. (1998) have been reported that soil myco-flora and fungal population of a particular soil significantly differ from season to season. Dkhar and Mishra (1987) revealed that the variations in fungal diversity in some soil types were due to changes in soil organic contents, pH, water holding capacity and temperature of respective season. According to Bhat and Kaveriappa, 2011; Gomathi *et al.*, 2011; Shiny *et al.*, 2013; Gopal & Kurien, 2013 species of the genus *Aspergillus* and *Penicillium* were dominant in soil that revealed the similarity with our present findings. The ability of *Aspergillus* species to produce toxins and antibiotics produced by *Penicillium* species may be helpful in inhibiting the growth of other fungal species.

Table.1 Soil Physico-Chemical Properties of the Rhizospheric Soil Samples Collected from the Rhizosphere of Five and Twenty Year Old Tea Plantations

Age (Years)	pH	E.C. **(**cScm-1)	M.C. (%)	W.H.C. (%)	B.D. (g/cm^3)	Clay (%)	Slit (%)	Sand (%)	O.C. (%)	N (ppm)	P (ppm)	K (ppm)	O.M.
5	4.56± 0.012	112± 0.33	20.5 ±0.06	47.02 ±7.67	1.28± 0.09	11± 1.56	23± 0.71	66± 2.81	1.49 ±0.28	58.12 ±2.52	10.11±0.49	41.8± 0.21	2.56 ± 0.96
20	4.51± 0.003	117± 0.51	29.5 ±.03	50.11 ±5.93	1.29± 0.15	12± 1.32	19± 0.87	69± 2.73	1.54 ± 0.03	66± 2.36	13.01±1.21	47.88 ±0.26	2.65 ± 0.84

Table.2 Showing Seasonal Percentage of Relative Abundance of Rhizospheric Microfungi Associated with Two Different Age Group of Tea Plantations

Fungal flora	Winter		Pre monsoon		Monsoon		Retreating Monsoon	
	5 Years	20 Years	5 Years	20 Years	5 Years	20 Years	5 Years	20 Years
Alternaria alternata	-	-	6.45	-	-	-	-	-
Alternaria tennius	-	-	2.85	2.70	-	-	-	-
Aspergillus candidus	-	-	-	8.75	6.60	8.50	2.77	6.81
Aspergillus clavatus	-	-	7.14	5.59	6.25	-	11.11	9.09
Aspergillus flavus	4.0	-	-	-	10.34	12.50	5.55	-
Aspergillus fumigatus	-	-	17.07	5.26	11.53	10.34	-	2.27
Aspergillus niger	8.00	10.52	16.48	16.45	15.38	22.80	16.66	18.18
Aspergillus vessicolor	16.66	5.6	-	-	-	-	-	2.27
Bispora sp	-	-	5.71	10.69	6.25	5.26	-	2.27
Botrytis sp	-	-	6.06	5.48	-	-	-	-
Candida ablicans	-	6.45	4.39	-	-	-	-	-
Cephalosporium ceremoiodes	-	-	5.77	7.31	9.37	8.50	11.11	2.27
Cladosporium herbarum	-	-	9.01	5.71	11.53	17.24	13.88	13.63
Culvularia geniculatum	-	3.22	9.04	8.57	3.12	7.89	-	-
Dendropsis sp	-	-	3.03	-	-	-	-	-
Fusarium oxysporium	-	-	2.85	5.26	9.37	-	8.33	11.36
Fusarium poea	-	-	-	7.89	-	-	-	2.27
Giocladium sp.	-	-	-	-	-	8.33	-	-
Giotrichum sp.	-	-	-	-	-	3.12	-	-
Helminthosporium oryzae	-	-	12.12	14.28	12.50	2.77	2.77	-
Humicola brevis	8.02	10.03	6.06	8.57	6.60	6.25	8.33	2.27
Nigrospora sp	-	-	3.03	2.85	-	-	-	-
Penicillum citrinum	5.33	6.45			-	-	-	-
Penicillium granulatum	9.9	13.33	9.37	5.55	-	-	-	6.81
Penicillium restrictum	26.13	5.96			-	-	-	-
Penicillium viridicatum	-	-	6.06	5.40	-	-	11.11	-
Rhizopus nigricans	6.16	15.36	6.06	2.85	7.69	10.74	5.55	-
Rhizopus sp	-	-	-	-	6.60	5.70	-	4.54
Trichoderma lignorum	19.85	13.14	-	-	10.00	6.25	-	6.81
Verticillium candilabrum	-	-	-	-	10.00	8.50	-	-
Verticillium effusum	6.00	6.45	12.12	8.57	15.38	10.34	2.77	4.54
Verticillium glaucam	9.09	13.33	6.25	10.81	3.84	3.14	-	2.27
White sterile hyphae	12.2	9.49	8.57	7.19	7.69	6.89	3.22	5.26

Figure.1 Graphical Representation of the Rhizospheric Soil Physiochemical Properties of Two Different Age Group Tea Plantations

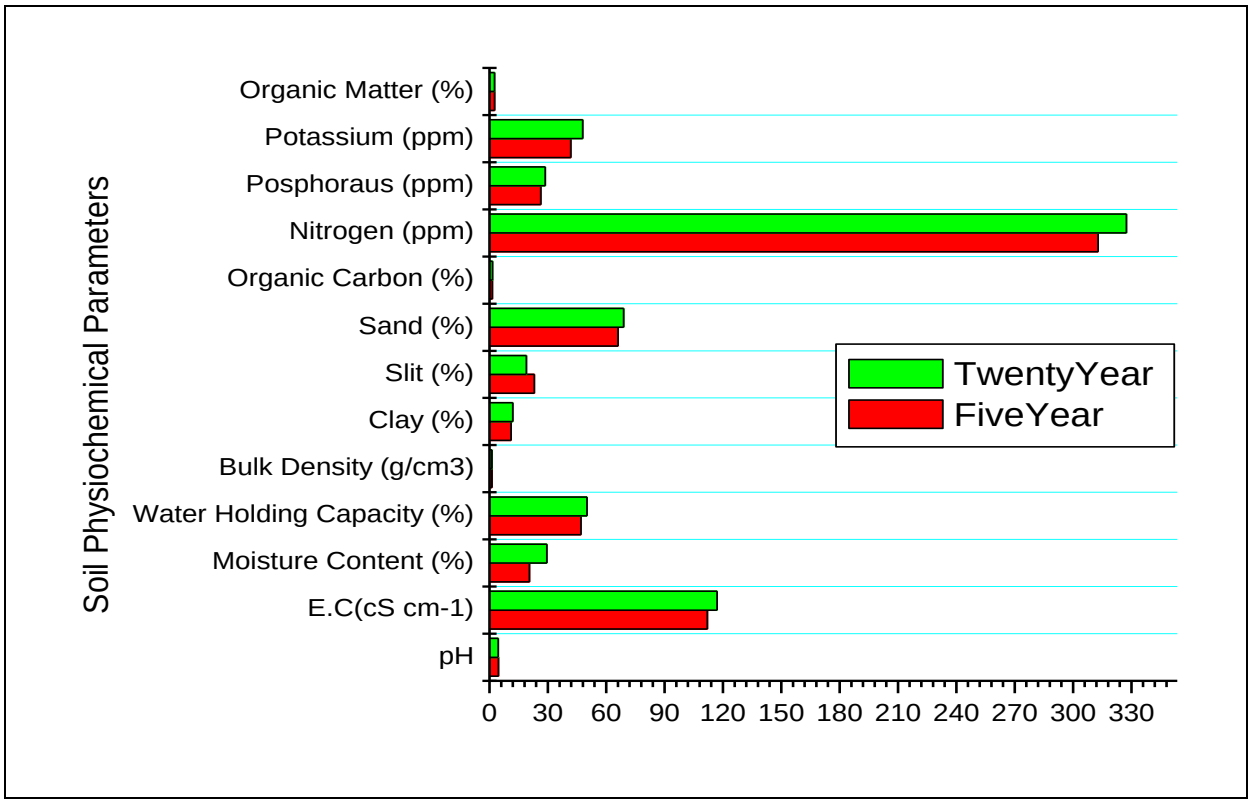


Figure.2 Graphical Representation of Relative Abundance (%) of Fungal Species Isolated from Two Different Age Group of Tea Plantations During Winter Season

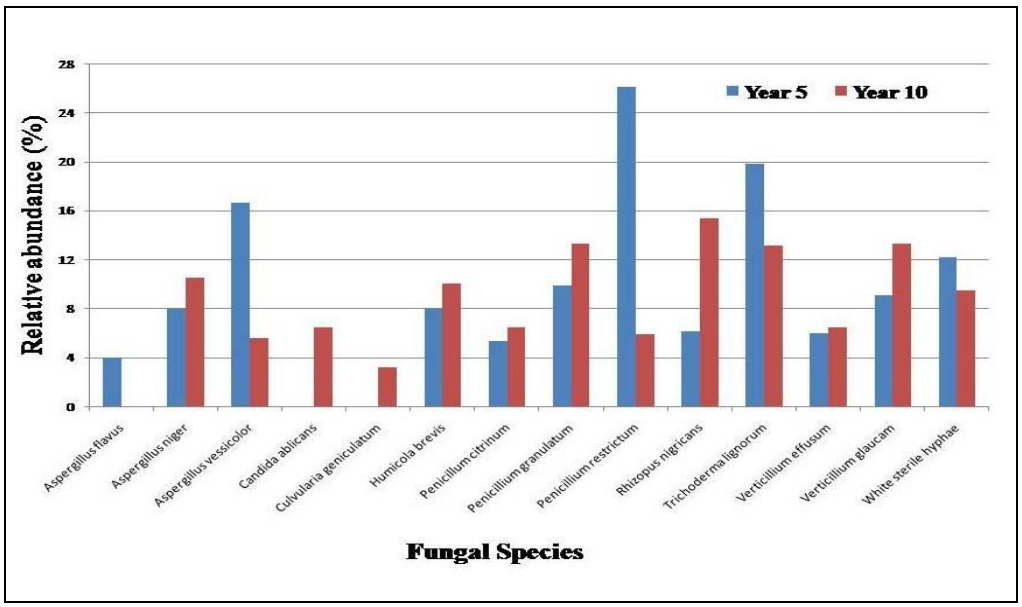


Figure.3 Graphical Representation of Relative Abundance (%) of Fungal Species Isolated from Two Different Age Group of Tea Plantation During Premonsoon Season

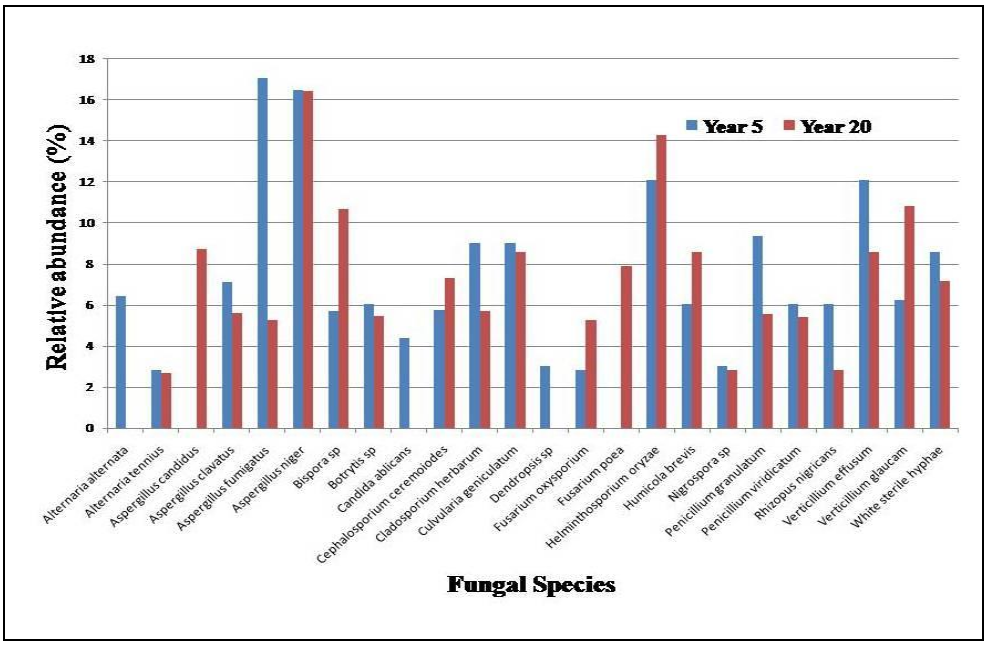


Figure.4 Graphical Representation of Relative Abundance (%) of Fungal Species Isolated from Two Different Age Group of Tea Plantation During Monsoon Season

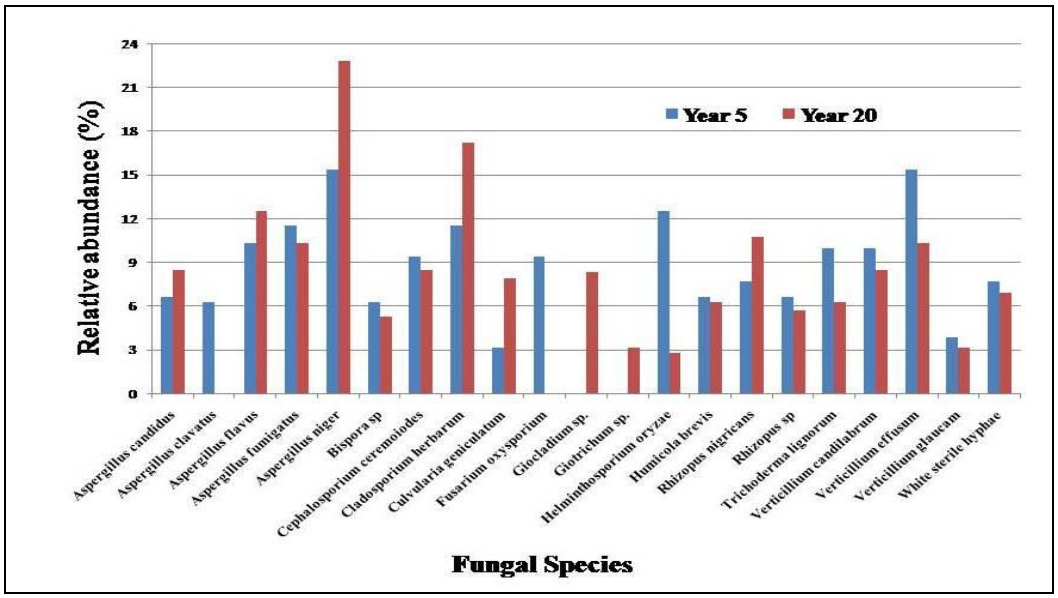
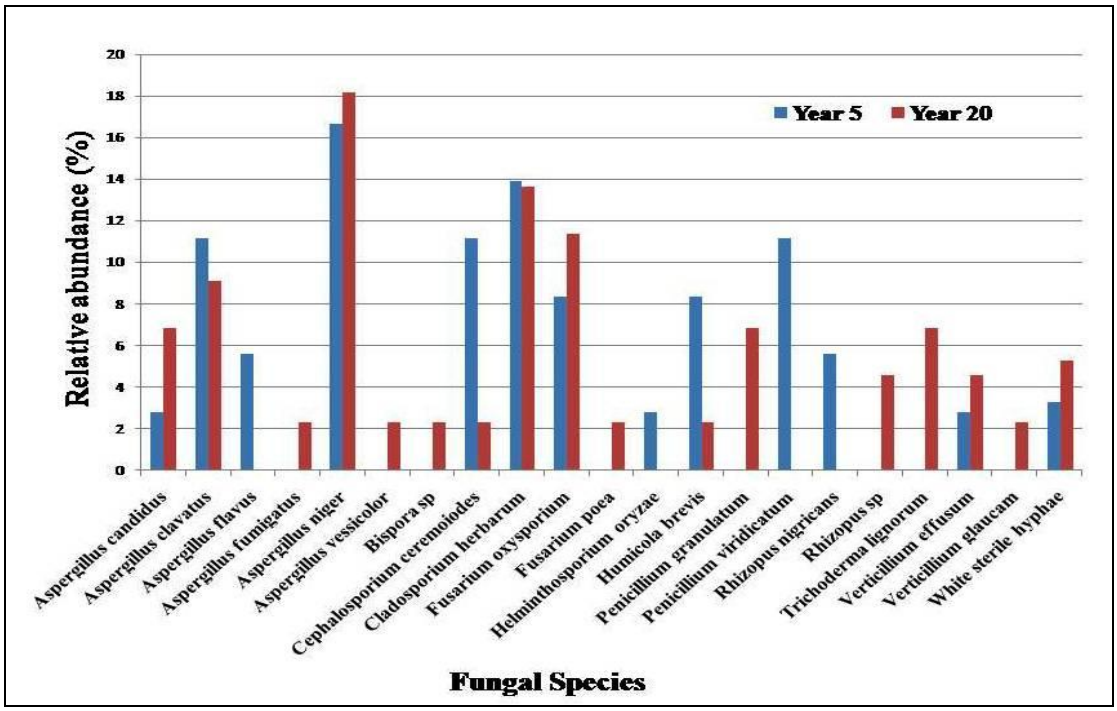


Figure.5 Graphical Representation of Relative Abundance (%) of Fungal Species Isolated from Two Different Age Group of Tea Plantation During Retreating Monsoon Season



In conclusion, the present investigation provides valuable information about the seasonal rhizosphere fungal diversity of two different age group of tea plantation. A total of 32 fungal species and white sterile hyphae were isolated and identified from both the selected plantation sites during the course of the investigation. Among the isolated rhizospheric microfungi *Aspergillus niger*, *Humicola brevis* and *Verticillium effusum* were isolated throughout the year indicating that the nutrient content and the environment of both the selected plantation sites are suitable for the growth of the said fungal isolates. The most dominant fungal isolates are belonging to the genus *Aspergillus and Penicillium* However; the present study has some limitation as the sampling of rhizospheric soil was confined only to selected experimental plots. There is need for a wider study area for the complete representation of the seasonal fungal diversity associated with the tea plantation.

Acknowledgement

We owe our special thanks to HOD, Department of Botany Govt. Degree College Dharmanagar for providing laboratory facilities to complete our research work.

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How to cite this article:

Datta, A., Chakraborty, K., Paul, C. and Sharma, B. K. 2016. Seasonal Diversity of Rhizospheric Microfungi in Two Different Age Group of Tea Plantation in Tripura, Northeast India. *Int.J.Curr.Microbiol.App.Sci.* 5(3): 363-372.
doi: http://dx.doi.org/10.20546/ijcmas.2016.503.043