J. Microb. World 13 (1) PP : 99-105, 2011
Microbiologists Society

STUDIES ON THE DIVERSITY OF RHIZOSPHERE MYCOFLORA OF SOME COMMON MEDICINAL PLANTS OF KALGACHIA, BARPETA, ASSAM.

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ABSTRACT

An attempt was made to determine the diversity of rhizosphere and non-rhizosphere mycoflora of three medicinal plants viz- *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb at different stages of growth in the year 2008. Analysis of rhizosphere and non-rhizosphere soil samples from the plants revealed that comparatively higher fungal populations was found in the rhizosphere soil as compared to non-rhizosphere soil. The physico-chemical properties of the soil samples collected from the field of the three plants were analyzed. A total of 13 different fungal species belonging to 10 genera were isolated from the rhizosphere and non-rhizosphere soil of *Centella asiatica* (L.) and *Bacopa monnieri* (L.) (Sandy-loam soil) while 11 different fungal species belonging to 9 genera were isolated from the rhizosphere and non-rhizosphere soil of *Houttuynia cordata* thunb (Clay-loam soil). The highest number of fungal colonies (333 number of colonies) were isolated from the rhizosphere and non-rhizosphere soil of *Centella asiatica* followed by *Bacopa monnieri* (240 number of colonies) and *Houttuynia cordata* (215 number of fungal colonies). The highest total fungal colonies (107 number of fungal colonies from *Centella asiatica,* 80 number of colonies from *Bacopa monnieri* and 74 number of colonies from *Houttuynia cordata)* were isolated from the rhizosphere soil when the plants attained flowering stage. The species of *Aspergillus* was found to be the dominant fungi amongst the other isolated fungi. Some of the important types of fungi isolated were *Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Penicillium oxalicum, P. rubrum, Rhizopus sp.* and *Trichoderma viride.*The physico-chemical properties of the soil such as soil P^H, temperature, moisture content, textural class, phosphorus, potassium, organic carbon and total nitrogen were found to play an important role on the composition and concentration of soil mycoflora.

KEY WORDS DIVERSITY, RHIZOSPHERE MYCOFLORA, MEDICINAL PLANTS AND ANTIMICROBIAL ACTIVITIES.

INTRODUCTION

Centella asiatica (L.) Urban is a widely available Indian herb belongs to the family Apiaceae. In traditional Indian system of medicine it has been used for the treatment of leprosy, bronchitis, asthma, syphilis, wound healing and as an antidote against cholera. The plant contains a variety of chemical substances and essential oil. The essential oil of *Centella asiatica* has some antibacterial activity (Oyedeji and Afolayan, 2005).

Bacopa monnieri (L.) Pennell is also a widely available Indian herb belongs to the family Scrophulariaceae. It has been used in indigenous Indian system of medicine as a nerve tonic and as a cure for epilepsy and insanity. The chemical compounds present in *Bacopa monnieri* (L.) are triterpenoid glycosides and triterpenoid saponin (Sivaramakrishna *et al*, 2005).

Houttuynia cordata thunb is a herb belongs to the family Saururaceae. It grows extensively in eastern and southern Asia as well as in India. The essential oil of aerial parts of *Houttuynia cordata* possesses antibacterial, antiviral, antifungal activities and it can also inhibit HIV-I and SARS (Ishtiaq *et al,* 2007).

Soil serves as a dynamic reservoir and habitat of a wide variety of a heterogeneous microorganism. The soil microorganisms carry out their diverse microbial activities not in association but by maintaining a active relationship with plant roots, soil substrates and other microbial species. Root systems of living plants excrete chemical compounds into the surrounding soils and thus the rhizosphere has important effect on the occurrence and composition of diverse microbial population of the soils (Aaron and Raymond, 1980). A large number of soil microbes survive together in the rhizosphere region of plants. The nature and activity of microorganisms in rhizosphere influence the growth and development of plant significantly (Anandapandian and Rajendran, 2003). Rangaswami (1988) reported that the metabolic activities of microorganisms in the rhizosphere is several folds higher than the soil further away from the influence of root zone. Rao and Reddy (1990) reported that fungal population in the rhizosphere and non-rhizosphere soil varied with types of crop and its age. Karthikeyan *et al*; (2008) studied the rhizosphere microbial diversity of some commercially important medicinal plants and they found more microbial population in the rhizosphere soil as compared to non- rhizosphere soil.

The information on the qualitative and quantitative nature of rhizosphere mycoflora of *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb. are not sufficiently available. Therefore, the present study was carried out to determine the diversity of soil fungi in the rhizosphere and non- rhizosphere soil of the medicinal plants at different stages of growth.

MATERIALS AND METHODS

The medicinal plants viz- *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb grown in different soil types were taken for the investigation. Rhizosphere and non- rhizosphere mycoflora were analyzed at the three different growth stages of the plants viz- seedling stage, flowering stage and fruiting stage. The different soil samples were collected from the experimental sites and the physico-chemical characteristics were analyzed. The soil P^H was determined in soil water suspension (1:2.5) using a digital P^H meter (Jackson, 1973), temperature was measured at month wise interval by inserting a soil thermometer into the soil and soil moisture was estimated by Gravimetric method. Textural class of the sampled soil was determined by International pipette method (Piper, 1966). Organic carbon percentage was estimated by Walkley and Black wet digestion method (Piper, 1966). Phosphorus was estimated colorimetrically employing Vanado-molybdate method. Potassium was estimated by using Flame photometer with neutral normal ammonium acetate solution (Stanford and English, 1949). Nitrogen was determined by Kjeldahl method using Kjeltech auto analyzer 1030 (Piper, 1966).

Rhizosphere mycoflora of the three medicinal plants were analyzed by Harley and Waid technique (1955). The root systems of the three medicinal plants from the experimental sites were

carefully excavated and aseptically taken to the laboratory. The root systems were then placed into 250 ml of conical flask containing 100 ml of sterilized distilled water. The roots were subjected to constant washing in sterilized distilled water. Suitable dilutions were prepared from the suspension and 1 ml soil suspension from the 10^{-4} dilutions was poured in sterilized petriplates containing 20 ml Czapeck's Dox agar medium with added tetracycline (5 mg/100 ml medium). Analysis of non-rhizosphere mycoflora was carried out by dilution plate method (Warcup, 1951). Non-rhizosphere soil samples were collected aseptically near the plants free from the influence of root systems and 1g soil was then transferred into 100 ml sterilized distilled water. Suitable dilutions were prepared and plating was done at 10^{-4} dilution using 20 ml Czapeck's Dox agar medium with added tetracycline (5 mg/100 ml medium). All the petriplates were incubated at 27 $\pm$ 1°C and the fungal colonies were counted on the 7th days of incubation.

Fungi were identified on the basis of their morphological and reproductive characters in accordance with the manuals of Gilman (1957) and Barnett and Hunter (1972).

RESULTS AND DISCUSSION

The experimental results (Table 2) revealed the number and types of fungi in the rhizosphere and non-rhizosphere soil with the progressive growth stages of *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb in different soil types. A total of 13 different fungal species were isolated belonging to 10 genera from the rhizosphere and non-rhizosphere soil of *Centella asiatica* (L.) and *Bacopa monnieri* (L.) and 11 different fungal species were isolated belonging to 9 genera from the soil of *Houttuynia cordata* thunb during the study period. Some of the important types of rhizosphere and non-rhizosphere fungi isolated from the soil of *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb were *Aspergillus flavus, A. fumigatus, A. niger, Curvularia lunata, Fusarium oxysporum, Mucor hiemalis, Penicillium oxalicum, P. rubrum, Rhizopus sp.* and *Trichoderma viride* while *Alternaria sp. Cladosporium sp. Helminthosporium sp. Nigrospora sp. Phoma sp.* and *Verticillium sp.* occurred in lesser number in both of the rhizosphere and non-rhizosphere soil of the plants. *Cladosporium sp., Penicillium citrinum, P. rubrum* and *Verticillium sp.* were only recorded in the rhizosphere and non-rhizosphere soil of *Bacopa monnieri* whereas *Nigrospora sp.* was only recorded in the soil of *Houttuynia cordata.* It was observed from the results (Tables 2) that the highest number of fungal colonies (333 number of colonies) were isolated from the rhizosphere and non-rhizosphere soil of *Centella asiatica* (Sandy-loam soil) followed by *Bacopa monnieri* (240 number of colonies, Sandy-loam soil) and 215 number of fungal colonies were isolated from the rhizosphere and non-rhizosphere soil of *Houttuynia cordata* (Clay-loam soil). The results show that the highest total fungal colonies (107 number of fungal colonies from *Centella asiatica,* 80 number of colonies from *Bacopa monnieri* and 74 number of colonies from *Houttuynia cordata)* were isolated from the rhizosphere soil when the plants attained flowering stage, while the lowest total fungal colonies (52 number of fungal colonies from *Centella asiatica,* 34 number of colonies from *Bacopa monnieri* and 31 number of colonies from *Houttuynia cordata)* were isolated from

Table 1 Physico-chemical properties of the soils collected from *Centella asiatica* (L.), *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb growing experimental fields.

Months	*Centella asiatica* (L.) and *Bacopa monnieri* (L.) fields.			*Houttuynia cordata* thunb field.		
	P^H	Temp. (°C)	Moisture (%)	P^H	Temp. (°C)	Moisture (%)
January'09	6.60	17.0	72.0	6.50	16.5	73.2
February	6.31	18.0	71.4	6.25	17.0	74.0
March	6.15	22.6	71.8	6.10	21.0	75.5
April	6.10	23.0	74.6	6.05	22.8	76.5
May	5.80	24.0	78.0	5.65	24.3	79.8
June	5.75	27.8	79.2	5.50	25.8	83.2
July	5.60	28.8	85.5	4.92	26.6	86.5
August	5.80	29.5	84.0	5.10	29.9	87.8
September	5.90	26.5	82.7	5.86	28.0	83.0
October	6.15	26.0	79.1	6.10	25.0	78.0
November	6.20	24.0	75.0	6.15	22.2	73.5
December	6.45	18.0	74.3	6.32	17.8	70.5

Textural class : Sandy-loam
P_2O_5 content (Kg ha^{-1}) : 19.50
K_2O content (Kg ha^{-1}) : 135.00
Organic carbon (%) : 0.83
Total Nitrogen (%) : 0.10

Textural class : Clay-loam
P_2O_5 content (Kg ha^{-1}) : 25.03
K_2O content (Kg ha^{-1}) : 128.35
Organic carbon (%) : 0.79
Total Nitrogen (%) : 0.08

the rhizosphere soil during seedling stage. *Aspergillus niger* showed the highest number of colonies in rhizosphere soil of *Centella asiatica* (18 number of colonies) and *Bacopa monnieri* (15 number of colonies) and *Fusarium oxysporum* (15 number of colonies) showed the highest number in the rhizosphere soil of *Houttuynia cordata* during the flowering stage. Some differences were observed in the number and types of fungi between the rhizosphere and non-rhizosphere soil amongst the three medicinal plants which is similar with the findings of Ali *et al*; (2007), Karthikeyan *et al*; (2008), Pandey and Upadhaya (2000) and Anandapandian and Rajendran (2003). A. gradual increase and proliferation of fungal population was observed during the advancing growth stages of the plants

which might be due to the gradual increase in exudations of some chemicals by plant roots, which is in agreement with the findings of Venketson and Rangaswami (1964) and Kagti (1966). It was observed from the results that the species of *Aspergillus* to be the dominant fungi in the rhizosphere soil of the three medicinal plants collected during the three growth stages. The result corroborate with the findings of Pandey and Upadhaya (2000) and Desai (1999). The variations of fungal population observed during the different growth stages of the three plants might be due to the variation in the physico-chemical properties of the soils collected from the

Table 2 : Number and types of fungi isolated from rhizosphere and non-rhizosphere soil of *Centella asiatica* (L.) *Bacopa monnieri* (L.) and *Houttuynia cordata* thunb plant at the three different growth stages (Results represent the average number of fungal colonies/mg soil).

Fungal types	Seedling stage						Flowering stage						Fruiting stage					
	RS1	RS2	RS3	NRS1	NRS2	NRS3	RS1	RS2	RS3	NRS1	NRS2	NRS3	RS1	RS2	RS3	NRS1	NRS2	NRS3
Alternaria sp.	3	-	2	-	-	-	2	2	3	1	2	-	3	2	2	-	-	-
Aspergillus sp.	3	-	-	-	-	-	3	-	-	1	-	-	2	-	-	1	-	-
A. flavus	-	4	-	-	3	-	-	8	-	-	3	-	-	5	-	-	2	-
A. fumigatus	6	-	3	2	-	1	12	-	6	5	-	3	8	-	4	4	-	3
A. niger	10	6	5	5	3	2	18	15	10	10	8	4	12	12	6	5	6	3
Cladosporium sp.	-	3	-	-	-	-	-	4	-	-	-	-	-	-	-	-	3	-
Curvularia lunata	5	-	3	3	-	3	10	-	8	4	-	5	8	-	5	5	-	2
Fusarium oxysporum	8	5	6	3	3	3	15	12	15	6	5	5	10	10	10	4	4	4
Helminthosporium sp.	-	-	1	2	-	-	3	-	3	-	-	-	-	-	2	-	-	-
Mucor hiemalis	-	3	3	-	1	2	-	6	8	-	3	4	-	4	4	-	2	3
Nigrospora sp.	-	-	-	-	-	1	-	-	3	-	-	-	-	-	-	-	-	2
Penicillium citrinum	-	-	-	-	-	-	-	5	-	-	2	-	-	3	-	-	-	-
P. oxalicum	8	-	4	5	-	2	13	-	12	5	-	5	10	-	8	5	-	3
P. rubrum	-	3	-	-	1	-	-	10	-	-	4	-	-	8	-	-	4	-
Phoma sp.	-	2	-	1	-	-	4	3	-	-	-	-	3	2	-	-	-	-
Rhizopus sp.	3	-	-	-	-	-	8	-	-	3	-	-	5	-	-	3	-	-
Trichoderma viride	4	2	-	3	2	-	12	7	-	4	3	-	8	5	-	5	2	-
Verticillium sp.	-	2	-	-	-	-	-	3	-	-	-	-	-	-	-	-	2	-
Dark sterile mycelia	-	2	2	-	-	1	3	3	3	2	1	1	2	2	2	-	-	-
Unidentified group	2	2	2	1	1	-	4	2	3	3	1	2	2	2	2	-	-	1
Total no. of fungal colonies	52	34	31	25	14	15	107	80	74	44	32	29	73	55	45	32	25	21
Percentage of total colonies	15.6	14.1	14.4	7.5	5.8	6.9	32.1	33.3	34.4	13.2	13.3	13.4	21.9	22.9	20.9	9.6	10.4	9.7

RS1= Rhizosphere soil of *Centella asiatica* (L.) **NRS1=** Non-rhizosphere soil of *Centella asiatica* (L.)
RS2= Rhizosphere soil of *Bacopa monnieri* (L.) **NRS2=** Non-rhizosphere soil of *Bacopa monnieri* (L.)
RS3= Rhizosphere soil of *Houttuynia cordata* thumb **NRS3=** Non-rhizosphere soil of *Houttuynia cordata* thumb

fields of the plants (Table 1). In the present investigation, the number and types of fungal population were found higher in the rhizosphere and non-rhizosphere soil collected from *Centella asiatica* and *Bacopa monnieri* (Sandy-loam soil) than the soil collected from *Houttuynia cordata* (Clay-loam soil). This finding is similar with the finding of Ali *et al*, (2007). Ali *et al*, (2007) studied the influence of root systems of Parthenium plant on soil fungi in different localities of Guwahati, Assam and they isolated a higher number and types of fungal species from the soil of Noonmati area (Sandy-loam soil) than Jalukbari area (Clay-loam soil). Limited number and types of fungal species were recovered from the rhizosphere and non-rhizosphere soil of *Houttuynia cordata* at the three different growth stages of the plants which may be due to the secretion of some antimicrobial chemical compounds by the root systems into the soil. Lu Ni *et al*; (2010) and Ishtiaq *et al*; (2007) have also reported that the chemical compounds present in *Houttuynia cordata* possess antibacterial, antifungal and antiviral activities. It may be stated that the factors such as P^H, temperature, moisture content of soil, phosphorus, potassium, organic carbon and total nitrogen of the soil have some significant role on the composition and concentration of soil mycoflora

ACKNOWLEDGEMENT

The authors are thankful to the Head of the Department of Botany, Nabajyoti College, Kalgachia, Barpeta for providing laboratory facilities.

REFERENCES

Aaron, L.M. and Raymond, A.W. (1980). Aspects of diversity measurement for microbial communities. *Appl. Environ. Microbiol.* **40**(3): 576-586

Ali, J., Ali, E., Islam, N. F. and Bora, K. N. (2007). Studies on the influence of root systems of Parthenium plant on soil fungi in different localities of Guwahati, Assam. *J. Mycopathol. Res.* **45**(1) : 40-44.

Anandapandian, K.T.K. and Rajendran, K. (2003). Assessment of microbial diversity in rhizosphere of tree species in South Tamil Nadu. *Nature Environ. Poll. Technol.* **2**(2): 153-156.

Barnett. H.L. and Hunter, B.B. (1972). *Illustrated genera of imperfect fungi*, 4th ed. Macmillan Publishing co., New York.

Desai, P. (1999). Rhizosphere microflora of Mulberry. *J. Mycol. Pl. Pathol.* **29** (1) : 113-114.

Gilman, J.C. (1957). A Manual of soil fungi, 2nd ed. Iowa State University press, USA.

Harley, J.L. and Waid, J. S. (1955). A method of studying active mycelia of living roots and other surface in the soil. *Trans. Mycol. Soc.* **38**(2) : 104-118.

Ishtiaq, C. M., Yang, W and Yiyu, C. (2007). Gas chromatographic / mass spectrometric analysis of the essential oil of *Houttuynia cordata* Thunb by using on column methylation with tetramethy-lammonium acetate (Dietary supplements Report). *Journal of AOAC International,* January 1 (2007).

Jackson, M. L. (1973). *Soil Chemical Analysis.* Prentice Hall of India Pvt. Ltd. New Delhi.

Kagti, L. C. (1966). Studies on the distribution of the microorganisms in the rhizosphere of wheat *J. Assam. Sci. Soc.* **9** : 48-66.

Karthikeyan, B; Jaleel, C.A; Lakshmanan, G.M.A. and Deiveekasundaram, M. (2008). Studies on rhizosphere microbial diversity of some commercially important medicinal plants. *Colloids and Surfaces B: Biointerfaces;* **62**(1):143-145

Lu Ni, Xi, Peng, Li and Liu, Wen-Zhe (2010). Structure, components and functions of secretory tissues in *Houttuynia cordata*. *J. Integ. Pl. Biol.* **49**(12):1734-1745.

Oyedeji, O.A. and Afolayan, A.J. (2005). Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. *Pharmaceutical Biology,* **43** (3) : 249-252.

Pandey, K. K. and Upadhaya, J. P. (2000). Microbial population of rhizosphere and non-rhizosphere soil of pigeon pea : Screening for resident antagonist and mode of mycoparasitism. *J. Mycol. Pl. Pathol.* **30** (1) : 7-10

Piper, C. S. (1966). *Soil and Plant Analysis.* Hans Publishers, Bombay.

Rangaswami, G. (1988). Soil plant microbes interrelationship. *Indian Phytopath.* **42**(2) : 156-172.

Rao, C. G. and Reddy, K. S. (1990). Studies on the influence of the rhizosphere of various crops on soil mycoflora. *Indian Phytopath.* **43** : 258-259.

Sivaramakrishna, C; Rao, C.V; Trimurtulu, G. and Vanisree, M. (2005). Triterpenoid glycosides from *Bacopa monnieri*. *Phytochemistry,* **66** (23): 2719-2728.

Stanford, S. and English, L. (1949). Use of flame photometer in rapid test of K and Ca. *Agron. J.* **41** : 446-447

Venketson, R. and Rangaswami, G. (1964). Studies on the microbial population of paddy soil influenced by moisture percentage in the crops. *Indian J. Exptl. Biol.* **3** :30-33.

Warcup, J. H. 1951. The ecology of soil fungi. *Trans. Brit. Mycol. Soc.* **34** : 376-339.