Biodiversity of Mycoflora In Rhizosphere and Rhizoplane of Some Medicinal Plants in Kanakapura, Ramanagar Dist, Karnataka

KEMPEGOWDA M. S[1], RABIN CHANDRA PARAMANIK[2]
[1, 2] *Department of Botany, Rural Degree College, Kanakapura, Bangalore rural, Karnataka.*

Abstract- ***The earth is home to a rich and diversity array of all living organisams, whose genetic diversity and relationship with one another and their physical environement constitutes biodiversity of living organisams from all sources and ecological complexes of which they arepart, which includes within the species and between the species. Many soil micro-organisms are able to solubilize the unavailable phosphorus, increase uptake of nitrogen and also synthesize growth promoting hormones including auxin. Rhizosphere and rhizoplane of most of the plants is always rich in various populations of microorganisms. The microorganisms, on a continuous scale forms parasitic to mutualistic association with plants. In present study, we isolated rhizosphere and rhizoplane fungi from tthree medicinal plants species viz., Phyllanthus amarus, Coleus forskohii, Adhathoda vasica All three species found to grow in wild Kanakapura, Ramanagar Dist, Karnataka. A total of Twenty-five species belongs to sixteen genera were isolated. Aspergillus, Fusarium, Penicillium, Alternaria, Cladosporium are the dominant genera observed during all three-month period. Number of incidences of rhizosphere mycoflora were decreased from January to March; may be due to the decreasing soil moisture level, which is triggered by raise in soil and atmospheric temperature.***

Indexed Terms- ***Fungi, medicinal plants, Roots, Seasonal variation, Soil moisture, Temperature.***

I. INTRODUCTION

Herbal plants are of immense medicinal value for drugs and pharmaceutical industries. The growth of the herbal plants also depends on the population of soil microorganism present in their rhizosphere and rhizoplane area of plants as these microorganisms constitutes one of the important biotic and ecological factors responsible for plant growth. Hiltner (1904) introduced the term rhizosphere for the soil zone just adjacent to plant roots and Clark (1949) introduced the term rhizoplane to denote the external plant root surface and the closely adhering soil particles and debris. Fungal population in the rhizosphere and rhizoplane areas show a qualitative change with age of plants and also along with changing environment. Lugo et al (2003). Moore et al. (2004) observed seasonal changes in the composition of Arbuscular mycorrhizal fungi. Study of rhizosphere and rhizoplane microorganisms associated with plants is important for understanding their ecological role in natural environment. The role of fungi in soil is extremely complex and is fundamental to the soil ecosystem (Bridge & Spooner, 2001). Soil microorganisms may stimulate, inhibit or completely suppress the growth of the soil borne pathogens; affect mineral nutrition uptake and plant growth (Katznelson et al. 1948). Microorganisms, which establish positive interaction with plant roots, play a key role in growth and development of plants (Cavaglieri et al. 2009). There are a number of plant growth-promoting fungi in rhizosphere region such as species of the genera Trichoderma, Penicillium, Fusarium, and Phoma, which have the ability to stimulate the plant immune responses (Sudisha et al. 2013). Rhizosphere region is characterized by greater microbial activity than the soil away from plant roots. Rhizospheric associations play a major role in physiological and biochemical properties owing to the growth and ecological fitness of the hosts (Krishnamurthy et al. 2008). These microbes act as frontline defense for roots against pathogens and thus, the rhizosphere microorganisms have greater potential as bio-control agent. The population and activities of rhizosphere microorganisms have therefore show significant effects on crop productivity. The present paper

includes the study of fungal biodiversity of rhizosphere and rhizoplane area of three wild medicinal plants, viz *Phyllanthus amarus, Coleus forskohii, Adhathoda vasica.*

II. MATERIALS AND METHODS

- Study area

The study was conducted in Kanakapura tq, is located 34 Km away from Ramnagra distict.To the South-East of kanakapur City Kebbahalli and Narayanapura reserved forest located is about 10km away from kanakapura, land mostly covered by southern tropical dry and southern tropical moist deciduous forests. The temperature in the study area varies from 20˚ C to 38˚ C. The average rainfall of the area is 1200 mm.

- Sample collection

Soil and plant samples were collected from Kebbahalli and Narayanapura reserved forest. Samples were taken from a depth of 0–15 cm in the sampling farms where a zigzag format of sampling was used. The plants were uprooted and separated into the root and shoot systems. Soil lumps attached to root surface were shaken gently to separate soil particles still adhering to root system and this forms the rhizosphere soil sample. The samples were kept separately in paper bags and carried to the laboratory. Samples were kept in a refrigerator at 4°C during the period of experimentation.

- Isolation and screening

Malt extract agar media was used to isolate the both rhizosphere and rhizoplane fungi (Mandeel 2002). Soil dilution plate method was used to isolate the rhizosphere fungi (Amin *et al.* 2007). For rhizoplane fungi isolation, the root segments were washed in slow running tap water for one hour, rinsed in sterile distilled water then they were surface disinfected with 0.5% Sodium hypochlorite solution for five minutes (Sayed & Morsy,2000) and once again washed with distilled water. The root samples were cut into one cm small segments, dried in sterile blotting paper (Youssuf et al. 2006) and inoculated to petriplates containing media, then incubated at 25˚C for 5 days (Amin et al. 2007). Colonies of fungi emerging from media were counted and identified using standard manuals (Barnett 1960, Ellis 1976, Subramanian 1983, Manoharachary, 2006). All the experiments conducted in three replicates.

- Results

Table 1. Average fungal incidences of rhizoplane sample in three months during 2019.

Fungal species	*Phyllanthus amarus*			*Coleus forskohii*			*Adhathoda vasica*		
	Jan	Feb	March	Jan	Feb	March	Jan	Feb	March
Alternaria alternata	5.2	4.5	2.25	4.5	3.5	1.75	2.5	2.25	2
Aspergillus flavus	5.5	4.0	2.25	5.5	4.5	3.25	5.75	6	1.5
Aspergillus fumigatus	6.0	5.25	4.5	7.25	5.5	3.75	4.25	5	1.75
Aspergillus niger	8.0	6.0	5.25	9	8.5	3	6.25	3.75	5
Aspergillus ochraceus	2.25	1	0.25	3	5.5	2.5	2.25	2.25	0.25
Aspergillus repens	0	0	0	0	1	0	0	0	0
Cephalotrichum sp.	2.0	1	0	0	1	0	1.25	2.25	1.25
Cercospora sp.	0	0	0	1	3	0	0	0	0
Chaetomium globosum	2.5	1	0.25	1.25	0.75	0.5	0.75	0.75	1.25
Cladosporium sp.	8	7.5	5.75	7	3.25	3.25	5.75	6.5	1.75
Curvularia lunata	2.5	1.75	1	3.75	2	3.25	0	0	0
Curvularia pallescens	*0*	*0*	*0*	0	0	0	0	0	1.75
Fusarium monoliforme	4.0	3.25	2.0	3.75	3.25	2.75	2.25	3	1.25
Fusarium oxysporum	6.75	5.25	3.75	6.75	4	4.5	6	4	3.75
Macrophomina phaseolina	1	0	0	1.5	0	0	0	0	0
Myrothecium roridum	0	0	0	0.75	0	0.5	0	0	0

penicilium glabrum	0	0	0	2.75	0	0	0	0	0
Penicillium digitatum	1.5	4.25	3.0	1.5	4.5	2.5	5	4.25	1.5
penicillium nigricans	1.75	2.75	0.25	1.25	1.75	0.25	3	2.75	0
Pestolotia macrotricha	0	0	0	0	0	0	0.75	0	0
Pestolotia sp.	0	0	0	0	0	0	0	0.25	0
Phoma sp.	0.50	0.25	0	0.75	1.75	0.25	1.5	1	0.25
Rhizopus nigricans	2.25	1.75	0	1.25	1.25	1.25	1.25	1.25	0
Trichoderma viridae	6.0	0	0	4.75	0	0	3.75	0	0
Verticillium sp.	0	0	0.25	0	0	0.5	0.25	1	0.5

Table 2. Average colonies of fungal incidences of rhizosphere sample in different dilutions from Three plant species.

Colonies of Fungal Species in different dilutions	*Phyllanthus amarus*				*Coleus forskohii*				*Adhathoda vasica*			
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Alternaria alternata	3.22	2.35	1.67	1.00	2.33	1.67	1.67	1.00	4.33	4.00	2.00	1.33
Aspergillus flavus	7.65	6.67	3.52	2.00	6.67	6.33	3.67	2.00	7.00	5.00	4.33	3.00
Aspergillus fumigatus	6.25	4.32	3.2	2.00	6.00	4.33	5.33	2.00	5.67	4.00	2.67	1.33
Aspergillus niger	5.0	7.55	4.50	2.25	4.00	8.00	4.67	2.00	5.33	4.33	3.67	2.00
Aspergillus ochraceus	6.25	6.0	3.00	2.27	6.00	5.00	2.67	1.00	2.00	2.00	2.33	-
Aspergillus repens	1.0	-	-	-	1.00	0.33	-	0.33	-	-	0.33	-
Cephalotrichum sp.	-	-	-	-	-	-	0.33	-2.33	2.33	2.67	1.00	0.67
Cercospora sp.	-	0.22	-	-	-	0.33	0.33	-	-	-	-	-
Chaetomium globosum	2.25	1.5	1.00	1.00	2.00	1.67	1.00	1.00	2.33	2.33	1.67	1.00
Cladosporium cladosporioides	3.50	4.50	2.33	1.33	3.67	5.00	2.33	1.33	5.00	3.67	1.67	0.67
Curvularia lunata	3.00	2.25	-	-	4.00	2.00	3.00	1.00	-	0.33	0.33	0.33
Fusarium monoliforme	6.00	5.25	2.33	1.00	5.00	5.00	2.33	2.33	5.00	2.67	2.33	1.00
Fusarium oxysporum	5.00	5.33	4.0	3.33	5.00	5.33	4.00	3.33	5.67	5.67	2.33	1.67
Macrophomina phaseolina	1.25	-	-	-	1.67	0.67	1.00	0.33	-	0.33	-	-
Myrothecium roridum	0.33	-	-	-	0.33	-	0.67	-	-	-	-	-
Penicilium glabrum	4.25	3.00	2.55	1.50	4.00	5.00	3.00	1.33	-	2.00	-	-
Penicillium digitatum	3.33	2.67	1.00	-	3.67	2.67	3.33	1.00	4.00	3.33	2.00	1.00
Penicillium nigricans	3.00	2.00	1.00	-	1.33	2.00	1.33	-	3.00	0.67	2.33	0.67
Pestolotia sp.	-	-	-	-	-	-	-	-	-	0.67	-	-
Phoma sp.	0.67	0.33	-	-	0.33	1.67	0.67	0.33	1.00	1.00	0.67	-
Rhizopus nigricans	2.67	1.67	-	-	1.33	1.00	1.67	-	3.00	2.67	1.67	-
Trichoderma viridae	3.5	1.67	1.00	-	4.00	1.67	2.33	1.67	1.33	1.33	1.00	0.67
Verticillium sp.	0.67	-	-	-	0.67	0.67	0.67	-	1.00	1.00	0.33	-

The rhizosphere and rhizoplane samples of *Phyllanthus amarus*, *Coleus forskohii* and *Adhathoda vasica* harbored twenty-five species belongs to sixteen genera. Rhizoplane sample showed maximum fungal incidence in all three plants (Table 1) when compared with rhizosphere sample (Table 2). Species of the genera *Aspergillus, Fusarium, Penicillium, Alternaria, Cladosporium,* showed higher incidence in both rhizosphere and rhizoplane. *Aspergillus* species, *Aspergillus niger* and *Aspergillus flavus* was found

abundantly during all the months. Whereas, *Aspergillus repens* was absent in January and March but it was found in February in the rhizoplane sample of *Phyllanthus amarus* and *Adhathoda vasica* .

The results also showed that *Phyllanthus amarus* and *Coleus forskohii* harbored higher occurrence of fungal communities such as, *Aspergillus, Fusarium, Penicillium,* compare to *Rhizopus, Cheatomium, Phoma* and *Cercospora. Myrothesium roridum* found in January but absent in February then again found in March both in rhizoplane and rhizosphere samples of *Coleus forskohii* but absent in *Phyllanthus amarus, Adhathoda vasica.*

In case of all three plants samples *Cladosporium, Aspergillus, Penicillium* found abundantly in three months. Whereas, *Trichoderma viridae* found only in January, absent in February and March. *Pestolotia* species occur in *Adhathoda vasica* but absent in other two plants (Table 1).

Rhizosphere sample of the all the three plants showed higher incidence in the 10^{-2} dilution, which gradually decreased to 10^{-5} dilution (Table 2). *Phyllanthus amarus* had very good rhizoplane and rhizosphere association when compared to *Coleus forskohii* and *Coleus forskohii* . All the three plant species showed higher incidence in January, which gradually decreased in February and March (Table 2).

DISCUSSIONS

Soil microbial population are immersed in a framework of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural system and natural system many of the factors that affect the fungal populations.

Similarly, Aspergillus flavus and A. niger were also dominates in the rhizosphere region. Gadgil (1965) also observed the colonization of hypomycetes fungi in the root region. Gherbawy et al. (2005) studied the seasonal variation of *Fusarium* species in rhizosphere of wheat field got maximum incidences of rhizosphere and rhizoplane microorganisms in January to February. They also reported that the number of fungal incidences decreases during the end of the winter in March. The present study of isolation of rhizosphere and rhizoplane fungi from *Phyllanthus amarus, Coleus forskohii* and *Adhathoda vasica* showed similar results with the exceptions of some species of fungi, which occurred or appears in March also. Increasing atmospheric temperature and decreasing soil moisture content from January to March may be the reason for decreasing fungal incidences. Das and Dkhar (2010) suggested that use of organic fertilizers increases the fungal population in the rhizosphere compared to control where no fertilizers were applied this shows the organic amendments are also positively affect the microbial growth in soil. *Aspergillus sulphureus, Penicillium islandicum* and *Paecilomy variotii* can effectively suppress the activity of *Pythium spinosum* (Sheikh & Abdelzaher, 2010). Several isolates of *Trichoderma viride was* selected for antagonistic screening against the fungal pathogens such as *Rhizoctonia solani, Sclerotium rolfsii,* and *Colletotrichum capsici* of *Vigna radiata* plant, by (Mishra *et al*. 2011) They showed that *Trichoderma viride* can effectively antagonize these pathogens.

REFERENCES

[1] Anitha, P., Lok, M. and Palni, S. 2007. The Rhizosphere effect in trees of the Indian central Himalaya with special reference to altitude. *Applied Ecology and Environmental Research* 5: 93-102.

[2] Barbara, M., Adam, O. and Boguslaw, C. 2008. Fungi isolated from the rhizosphere of spring cruciferous plants. *Acta Mycologica* 43:181-191.

[3] Barnett, H.L. 1960. *Illustrated genera of Imperfect fungi*. Second Edition. Minnea-polis, Burgess Publishing.

[4] Bibhuti, B.D. and Dkhar. 2010. Rhizosphere microflora of soybean as affected by organic amendments in Meghalaya. *NeBIO* 1: 1-7.

[5] Bridge, P. and Spooner, B.M. 2001. Soil fungi: diversity and detection. *Plant and soil*. 232: 147-154.

[6] Clark, F.E. 1949 Soil microorganisms and plant roots Adv. Agron. 1:24-88

[7] Ellis, M.B. 1976. *More Dematiaceous hypomycetes*. Kew Commonwealth Mycological Institute.

[8] Gherbawy, Y.A.M.H, Thanaa, A., Magharaby and. Yassmin, M. 2006. Seasonal variation of Fusarium species in wheat fields in Upper Egypt. *Archives of Phytopathology and Plant Protection*. 39: 365-377.

[9] Hiltener, L.. 1904Uber neure Erfahrungen und problem auf dem Gebiet der Boden bacteriologie und under leasonderer Berucksishtigung der Girunandungung und Brache Arbdtsch Lendeo Geo. 98:56-78.

[10] Katznelson, H., Lochhead, A.G. and Timonin, M.I. 1948. Soil microorganisms and the rhizosphere. *The Botanical Review.* 14: 543-554.

[11] Krishnamurthy, Y.L., Shankar, B.L. and Shashikala, J. 2008. Fungal communities in herbaceous medicinal plants from Malnad region, Southern India. *Microbes and Environments.* 23: 24-28.

[12] Lilia, C., Julieta, O. and Miriam, E. 2009. Rhizosphere Microbial Community structure at different Maize plant growth stages and root locations. *Microbial Research.* 164: 391-399.

[13] Lugo, M.A. Gonzalez Maza, M.E. and Cabello, M.N. 2003 Arbuscular mycorrhizal fungi in a mountain grassland II: Seasonal variation of colonization studied, along with its relation to grazing and metabolic host type Mycologia. 95(3): 407–415

[14] Mishra, B.K., Mishra, R.K., Mishra, R.C., Tiwari, A.K., Yadav, R.S. and Dikshith, A. 2011. Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in *Vigna radiate* L. *Scholars Research Library.* 3: 361-369.

[15] Moore, M., Opik, M. and Sen, R.(2004) Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common Pulsatilla species Funct. Ecol. 1(4) 554-562.

[16] Nagamani, I.K., Kunwar and Manoharachary, C. 2006. *Hand Book of soil fungi*. Published by I.K. International Pvt. Ltd. New Delhi.

[17] Nur, A.E.A., Abdulmoneium, M.A. and Saadabi. 2007. Contribution to the Knowledge of soil fungi in Sudan Rhizosphere mycoflora of Sugar cane at Kenna Sugar Estate. *International Journal of Botany.* 3: 97-102.

[18] Qaher, A.M. 2002. Micro fungal community associated with rhizosphere soil of *Zygophyllum qatarense* in arid habitats of Bahrain. *Journal of Arid Environments* 50: 665-681.

[19] Sayed, E.L.M. and Morsy, E.L. 2000. Fungi isolated from the endorhizosphere of halophytic plants from the Red Sea coast of Egypt. *Fungal Diversity*. 5: 43-54.

[20] Sheik, H.A. and Abdelzaher, H.M.A. 2010. Isolation of *Aspergillus sulphurous, Penicillium islandicum,* and *paecilomyces variotii* from agricultural soil and their biological activity against *Pythium spynosum,* the Damping-Off organism of soybean. *Journal of Biological Sciences.* 10: 178-189.

[21] Subramanian, C.V. 1983. *Hypomycetes Taxonomy and Biology*. Academic Press London Vol. 1 and 2**.**

[22] Sudisha, J., Mostafa, A., Lam, S.P.T. and Ito, S.I. 2013. Characterization of rhizosphere fungi that mediate resistance in tomato against bacterial wilt disease. *Journal of Experimental Botany* 64: 3829-3842.