Diversity of Mycoflora in Root and Rhizosphere Regions of *Alloteropsis Cimicina* (L.) Stapf

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Abstract
Endophytic fungi described as the internal mycota of living plants. The present study is an attempt to document mycoflora diversity of root, rhizosphere, bulk soil of grass *Alloteropsis cimicina* (L.) Stapf a less known species from North East India in Bhadra reserve forest region in two different seasons. Isolation, fungal community from collected samples by Moist blotter method, Potato dextrose agar (PDA) method, Malt extract agar (MEA) method Statistical analysis were done and the mean data by Simpson Diversity Index, Shannon Evenness Shannon Diversity Index (H1), Simpson Evenness, Colonization frequency and Jaccard’s Similarity Coefficient is measured. There were 700 fungal colonies belonging to 18 species of 11 genera and four NSF isolated.

Introduction
Endophytic fungi described as the internal mycota of living plants (Stone et al., 2012). Enhanced growth of infected plants also occurred in perennial ryegrass. Endophyte infected seeds also contain high concentrations of alkaloids and are less likely to be eaten by vertebrate and invertebrate seed feeders (Clay, 1990). Endophyte infection protect host from pests. The production of plant hormones and growth regulators appears to be an important mechanism by which fungal endophytes improve plant growth and yield under stressful conditions (Ellouze et al., 2014). *Beauveria bassiana* protected cotton and tomato plant against pathogens *Rhizoctonia solani* and *Pythium myriotylum* (Ownley et al., 2008).

Most hosts of clavicipitaceous endophytes are grasses (Clay, 1990). Rhizoplane and rhizosphere endophytic study on grasses of Panicoideae and Chloridoideae were carried out in Lakkavalli region of Karnataka (Vasanthakumari and Shivanna, 2011). The present study is an attempt to document mycoflora diversity of root,
rhizosphere, bulk soil of grass *Alloteropsis cimicina* (L.) Stapf a less known species from North East India in Bhadra reserve forest region in two different seasons (October-February 2019).

**Material and Methods**

In and around of Bhadra Reservoir Project was taken as study area, which is located at a distance of 29 km from Shivamogga (latitude 13° 72° 978° and longitude 75° 62° 862°). Two study sites were taken, in each three quadrate (2x2m) representing three replicates were established. Specimens were uprooted carefully from the soil The root system without soil particles was considered as the root sample and the soil samples around the root was considered as rhizosphere and soil sample with no plants growing was the bulk soil.

Roots were surface disinfected Rhizosphere and bulk soil samples were subjected to serial dilution at dilution 104 with sterile water (Vasanthakumari and Shivanna, 2011). Isolation, fungal community from collected samples by Moist blotter method, Potato dextrose agar (PDA) method, Malt extract agar (MEA) method. Fungal species are identified using standard manuals. (Barnets, 1960; Funder, 1961; Booth, 1971; Barnett and Hunter, 1972)

Statistical analysis were done and the mean data by Simpson Diversity Index Shannon Evenness Shannon Diversity Index(H1 ) Simpson Evenness,Colonization frequency. To evaluate the degree of community similarity of fungi associated between two regions and sampling seasons Jaccard's Similarity Coefficient is measured.

**Result**

There were 18 species of fungi belonging to 11 genera and four NSF isolated by three methods Among the emerged mycoflora some ascomycetes fungal species are specific to rhizosphere soil they are *Colletotrichum dematium, Myrothecium roridum, Pseudonectria foliicola* and a zygomycetes fungi *Mucor heimalis* is observed exclusively in rhizosphere. *Colletotrichum dematium* and *Myrothecium roridum* are observed only in first location and *Mucor heimalis* and *Pseudonectria foliicola* observed in second location.

Root contributes only a few fungal isolates of six species of four genera belonging to anamorphic ascomycetes and three non sporulating fungi The rhizosphere fungal communities were determined at the dilution of 10-4 since more number of fungal species occurred in this dilution. There were 17 anamorphic ascomycetes fungal species of 10 genera and one zygomycota with four NSF isolated. The bulk soil fungal communities were determined at the dilution of 10-4 since more number of fungal species occurred in this dilution. There occurred 11 fungal species of eight genera of anamorphic ascomycetes one zygomycota was isolated.

Anamorphs of acomycetes, zygomycetes and morphotypes frequency is more in PDA media than MEA. (62.79, 0.95 and 35.16 cfu g-1 respectively). Anamorphs of acomycetes the frequency of occurrence is more in location 2 (78.39 cfu g-1) than location 1. But in morphotype and zygomycetes it is more in location1 (41 and 1.9 cfu g-1 respectively) The species *Cladosporium herbarum* is having more colonization frequency value among all species in both PDA and MEA medias and also in location 1(21.5, 22.4 cfu g-1 and 21.4 cfu g-1 media respectively)

Diversity of mycoflora in second location more than first location. MEA media possess much diversity than PDA but some data showing more results in PDA and MB and evenness is more in MB. The rhizosphere soil has more fungal diversity than that of bulk soil and root. Jaccard’s similarity index of media PDA and MEA is 0.78 and Jaccard’s similarity index of location 1 and location 2 is 0.63. The fungal colonies observed in root and bulk soil is more similar. But in between bulk soil and rhizosphere it is less.
Table: Species Richness, Diversity and Evenness Indices of Fungal Communities Occurring in Different Locations, Plant Parts and Incubation Methods of *Alloteropsis Cimicina*

| Sample unites | Species richness | Diversity indices | Evenness indices | Total No. of isolates |
|---------------|-----------------|------------------|-----------------|----------------------|
|               |                 | Shannon diversity (H') | Simpson's diversity (D') | Shannon evenness (J') | Simpson's evenness (E') |
| Loc 1\(^2\)  | 16              | 2.18             | 5.25             | 0.80                 | 0.35                 | 218                     |
| Loc 2\(^3\)  | 15              | 2.07             | 6.03             | 0.74                 | 0.37                 | 548                     |
| PDA\(^4\)     | 19              | 2.17             | 6.03             | 0.73                 | 0.31                 | 310                     |
| MEA\(^5\)     | 16              | 2.24             | 7.17             | 0.80                 | 0.44                 | 348                     |
| MB\(^6\)      | 3               | 0.96             | 2.32             | 0.87                 | 0.07                 | 12                      |
| Root\(^7\)    | 9               | 1.82             | 4.92             | 0.83                 | 0.54                 | 235                     |
| Rhizosphere\(^8\) | 23          | 2.38             | 8.11             | 0.60                 | 0.35                 | 514                     |
| Bulk soil\(^9\) | 15           | 2.22             | 7.41             | 0.82                 | 0.49                 | 143                     |

Data based on the values for two study sites and two seasons in two trials; 2 & 3 Data are the average of three replicates; 4, 5 & 6 Data are the average of three replicates of two seasons in Potato Dextrose Agar, Malt Extract Agar and Moist Blotter methods; 7 Data are the average of of 210 root segments of the grass; 8 & 9 Data are the average of three replicates two seasons.

**Discussion**

The present study on diversity of mycoflora contributed around 700 fungal colonies belonging to 18 species of 11 genera. One zygomycetes species and four non-sporulating fungi were also observed. Rhizosphere shows maximum fungal diversity at the dilution of 10\(^{-4}\). The study shows that five species occurred in both soil and root, so that they are moving from soil to root. Considerable diversity of mycoflora was observed in the present study.

**References**

1. Abdel-Hafez S.I.I. 1982. Rhizosphere and rhizoplane fungi of *Triticum vulgare* cultivated in Saudi Arabia. Mycopathologia, 78: 79-86.
2. Aletaha R., A.A.S. Sinegani and D. Zafari. 2018. A survey on endophytic fungi within roots of Chenopodiaceae species under different environmental conditions. Mycosphere, 9(4): 618–634.
3. Ananda K. and K.R. Sridhar. 2002. Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. Canadian Journal of Microbiology, 48: 871-878.
4. Bhat K.G. 2014. Flora of South Kanara. Taxonomy Research Centre, Department of Botany, Poornaprajna College, Udupi.
5. Bhat K.G. and Nagendran C.R. 2001. Sedges and Grasses (Dakshina Kannada and Udupi districts). Dehra Dun.
6. Bhat P.R. and K.M. Kaveriappa. 2011. Rhizoplane mycoflora of some species of Myristicaceae of the Western Ghats, India. Tropical Ecology, 52(2): 163-175.
7. Chowdheri H.J. K.L. Garg and A.K. Jaitly. 1982. Occurrence of fungi in rhizosphere, rhizoplane and non-rhizosphere zones of some mangroves. Indian Journal of Marine Sciences, 11: 138-142.
8. Clay K. 1990. Fungal endophytes of grasses. Annu. Rev. Ecol. Sys., 21: 275-297.
9. Dawar S., M. Batool, M. Tariq and M.J. Zaki. 2014. Mycoflora in the rhizosphere of some wild plants around Karachi University Campus. Pak. J. Bot., 46(1): 369-373.
10. Eze C.S and J.E. Amadi. 2013. Rhizoplane mycoflora of cocoyam [Colocasia esculenta (L.) Schott] corms at nsukka, nigeria and their involvement in rot development during storage. Scientia Agriculturae, 4(3): 70-73.
11. Eze, C. Simon and A.J. Ezejiofor. 2014. Studies on rhizosphere and rhizoplane microflora of tomato (Lycopersicon esculentum Mill) seedlings. International Journal of Engineering Sciences & Research Technology, 3(7): 666-672.
12. Gizaw B., Z. Tsegay, G. Tefera, E. Aynalem and A. Tesfaye. 2018. Rhizosphere fungi identified from Poaceae and Cyperaceae family grass in north and east Showa: Ethiopia. Advances in Agricultural Technology & Plant Sciences, 1(2): 1-8.
13. Gopal K.S. and S. Kurien. 2013. Fungal diversity in the rhizosphere of tropical homestead and plantation crops of Kerala. International Journal of Agriculture, Environment & Biotechnology, 6(2): 249-253.
14. Linderman R.G. 1988. Mycorrhizal interactions with the rhizosphere microflora: The mycorrhizosphere effect. Phytopathology, 8(3): 362-371.
15. Manzelat S.F. 2017. Rhizosphere and rhizoplane mycoflora of date palm (Phoenix dactylifera) from Saudi Arabia. International Journal of Plant & Soil Science, 20(6): 1-11.
16. Mishra R.R. 1967. Nature of rhizosphere fungal flora of certain plants. Plant and Soil, Springer, pp. 162-166.
17. Nallanchakravarthula S., S. Mahmoud, S. Alstrom and R.D. Finlay. 2014. Influence of soil type, cultivar and Verticillium dahliae on the structure of the root and rhizosphere soil fungal microbiome of strawberry. PLOS One, 9(10): 1-10.
18. Olahan G.S., I.O. Sule, T. Garuba and Y.A. Salawu. 2016. Rhizosphere and non-rhizosphere soil mycoflora of Corchorus olitorius (Jute). Science World Journal, 11(3): 23-26.
19. Olahan G.S., T. Garuba, F.O. Ibrahim and S.B. Adeyemi. 2015. Isolation and identification of fungi associated with the rhizosphere and rhizoplane of tobacco (Nicotiana tabacum). Jewel Journal of Scientific Research (JJSR), 3(1): 128-131.
20. Olanrewaju S.O., E.B. Akinro and O.A. Oladipupo. 2014. Fungi colonization of the Rhizoplane of Okra (Hibiscus esculentus) plant. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 8(2): 18-22.
21. Ownley B.H., M.R. Griffin, W.E. Klingeman, K.D. Gwinn, J.K. Moulton and R.M. Pereira. 2008. Beauveria bassiana: Endophytic colonization and plant disease control. Journal of Invertebrate Pathology, 98(3): 267–270.
22. Papavizas G.C. and C.B. Dave. 1961. Extent and nature of the rhizosphere of Lupinus. Plant and Soil, 14(3): 215-236.
23. Rodriguez R.J., J.F. White, A.E. Arnold and R.S. Redman. 2008. Fungal endophytes: diversity and functional roles. New Phytologist, 182: 314–330.
24. Ruotsalainen A.L., H. Väre and M. Vestberg. 2002. Seasonality of root fungal colonization in low-alpine herb. Mycorrhiza, 12: 29–36.
25. Shaikh M.N. and D.N. Mokat. 2000. Bioactive metabolites of rhizosphere fungi associated with Cymbopogon citratus (DC.) Stapf. Journal of Pharmacognasy and Phytochemistry, 2: 32-38.
26. Shannon C.E. and W. Weiner. 1963. The Mathematical Theory of Communication. University of Illinois Press, Champaign IL, pp. 320.
27. Shivanna M.B. and M.M. Vasanthakumari. 2011. Temporal and spatial variability of rhizosphere and rhizoplane fungal communities in grasses of the subfamily Chloridoideae in the Lakkavalli region of the Western Ghats in India. Mycosphere 2(3): 255–271.
28. Simpson E.H. 1949. Measurement of Diversity. Nature, 163: 688.
29. Singh B.K., N. Nunan, K.P. Ridgway, J. McNicol, J. Peter, W. Young, T.J. Daniell, J.I. Prosser and P. Millard. 2008. Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. Environmental Microbiology, 10(2): 534–541.
30. Sobhy I.I. Abdel-Hafez, MA. Ismail, N.A. Hussein and N.A. Abdel-Hameed. 2012. Fusaria and other fungi taxa associated with rhizosphere and rhizoplane of lentil and sesame at different growth stages. Acta Mycologica, 47(1): 35-48.
31. Srivastava V. and A. Kumar. 2013. Biodiversity of mycoflora in rhizosphere and rhizoplane of some Indian herbs. Biological Forum – An International Journal, 5(2): 123-125.
32. Stone J.K., J.D. Polishook and J.F. White. 2012. Endophytic fungi. ResearchGate, pp. 241-270.
33. Ubogu M. 2013. Assessment of root zone mycoflora of three Hevea brasiliensis (Rubber) clones at Akwete plantations and their in vitro growth inhibition of Rigidoporus lignosus. European Journal of Experimental Biology, 3(2): 618-623.
34. Vasanthakumari M.M. and M.B. Shivanna. 2011. Fungal assemblages in the rhizosphere and rhizoplane of grasses of the subfamily Panicoideae in the Lakkavalli region of Karnataka, India. Microbes Environ., 26(3): 228-236.
35. Vasanthakumari M.M., G.E. Mallikarjunaswamy, K.G. Bhat and M.B. Shivanna. 2010. Grass species of Bhadra Wildlife Sanctuary in Karnataka, India. Indian Journal of Forestry, 33: 275–284.
36. Yadav S.R. 2010. Know Your Grass Genera through Hand Lens. Shivaji University, Kolhapur

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