Volume : 4 | Issue : 6 | June 2015 • ISSN No 2277 - 8179

Research Paper

"Seasonal and Spatial Variation of Microbial Population In the Rhizosphere Soils of Five Traditionally Important Medicinal Plant Species in Ne India"

Microbiology

KEYWORDS : Actinomycetes, bacteria,Coptisteeta, fungi, medicinal plant, rhizospheric soil

Chumi Khanikar — Department of Forestry, North Eastern Regional Institute of Science & Technology (Deemed University). Nirjuli-791 109, Arunachal Pradesh, India.

Sorokhaibam Sureshkumar Singh — Department of Forestry, North Eastern Regional Institute of Science & Technology (Deemed University). Nirjuli-791 109, Arunachal Pradesh, India.

ABSTRACT

Therhizospheresoils of five traditionally important medicinal plants were studied for a period of two years spreading over two seasons, summer warm and rainy season and winter cold and dry season. Fungal population, bacterial and actinomycetes population were recorded maximum during summer seasons while minimum were recorded during winter season. Bacterial population was highest (37 to 63x 106 CFU/g dry soils) followed by fungi (17to 33x 103 CFU/g dry soils) while lowest population was recorded foractinomycetes (5 to 17x105 CFU/g dry soils). Analysis of variance revealed significantly higher influence of time in terms of seasonal variation on the distribution of population of the three groups of microorgansms as compared to the plant species. The rhizosphere soils of Clerodendrumcolebrookianum and Asparagus racemosus displayed highest fungal population during summer seasons. Actinomycetes population was recorded lowest in the rhizosphere soil of Coptisteeta. The primary reason for variation in the distribution of bacterial population could be due to soil moisture content as revealed by significant positive correlations. Other microclimatic parameters such as soil temperature, nutrient availability and types of exudates released by plant roots could also play key roles in the distribution of microbial populations in the rhizosphere soils of medicinal plants.

INTRODUCTION

Microorganisms in soil are known to occur both in the bulk soil region where there is no growth of plants as well as in the rhizosphere region with profound effects of plants root systems. The population and diversity of these organisms are reported to be higher in the rhizosphere regionswhere active interaction occurs between microorganisms and the root systems than in bulk soil regions (Brimecombe, 2001; Yang et al., 2013). The distribution and intensity of rhizosphere microbial communities are reported to differ between plant species, within species and between different developmental stages of a given plant due to physiological effects (Garbevaet al., 2008; Broecklinget al., 2008; Batten et al., 2006 and Mougelet al., 2006). The main reasons for such variations are due release of various types exudates by plant root systems, heterogeneous nature of soil and climatic parameters. The exudates from plant root contain simple carbon substrates and various primary metabolites such as sugars, amino acids and organic acids in addition to a diverse array of secondary metabolites (Jones et al., 2004). The type of exudates and their abundance in rhizosphere region of different medicinal plant are reported to influence and harbor selective group of microbial communities (Broecklinget al., 2008; Girlanda et al., 2005; Innes et al., 2004).Similarly,temporal variations in soil physico-chemical properties pH, moisture, total organic matter and total nitrogen availability are reported toinfluence the population status and their species composition of microorganismsin the soil(Bhattacharyya and Jha, 2011; Das and Dkhar, 2011).In addition to these factors, climate variables such as temperature regime and rainfall are also known to have a profound effect on distribution and population structure of soil microorganisms.

Several studied have enumerated the bacterial, fungal and actinomycetes population ofrhizosphere of medicinal plants. Karthikeyan*et al.,* (2008) studied on rhizospheric soil of *Ocimum sanctum, Coleus forskholii, Catharanthusroseus* and *Aloe vera* in order to identify the populationof rhizosphere microorganism.Shilpkar*et al.,* (2010) investigated the dominance of different types of microbial communities at different monsoon seasons in rhizospheric soils of *Aeglemarmelos* tree.Tamilarasi*et al.,* (2008) have studied on root associated microbial populationon 50 locally available medicinal plants. North east India is known for rich medicinal plant resources of which *Asparagus racemosus* Willd.(Satmul), Tinosporasinensis (Lour.)Merr (Sagunilota), *Clerodendrumcolebrookianum*Walp. (Nephaphu), and *Vitexnegundo* L. (Posotia). *Coptisteeta* Wall. (Misimiteeta) are some traditionally important wild medicinal plants. These plants have their significance in traditional health systems as well as in pharmaceutical industries in addition to local economy.Present study has been done to investigate the distribution pattern of microorganism in the rhizosphere soil of these five medicinal plants.

MATERIALS AND METHODS

Study site selection

The present study was conducted by collecting soil samples from the rhizosphere of five selected medicinal plants found in two study sites, Dhakuakhana sub-division (27°14'16.68"N, 94°06'21.59"E, Altitude 102m amsl) ofLakhimpurlocated in Assam and Mayodia along Mishmi Hills range of Lower Dibang Valley district (28°14'37"N, 95°55'35"E, Altitude 2500 m amsl) of Arunachal Pradesh states, North East India.

Dhakuakhanais a subtropical humid (80 to 95% humidity) area with annual average temperature ranged between 6°C to 36°C. The average rainfall in the site was 3044 mm.Mayodiain Arunachal Pradesh is a complex hill terrain of varying elevations which receives heavy rainfall reaching upto5000 mm annually in the foothill areas. The vegetation in this site is dominated by temperate broad leaved forests. Annual temperatures range from below 0°C during winter and 36°C during summer.

Soil sampling and analysis

A minimum of 27 (9x3) random soil samples were collected from the rhizosphere region with the medicinal plant species.Fresh samples were processed in the laboratory and used for analysis of physic-chemical properties and microbial population studies.

Physico-chemical properties of soil

Soil texture was determined by a Boyuocos soil hydrometer on air dried samples method given by Okalebo*et al.*(1993) .Soil pH was determined by (1:2.5; soil water suspension) by a pH meter (Eutech). Soil temperature (ST) of the respective study locations was recorded with the help of a thermometer.Moisture contents (MC), total organic carbon (TC), total nitrogen (TN), total phosphorus (TP) were determined according to Okalebo*et al.,* (1993).

Microbial population analysis

Microbial populations were enumerated from the rhizospheric

soil of selected medicinal plants through culture dependent method, following the serial dilution technique. 10 g fresh soil was suspended in 90 ml sterile water and thoroughly shaken for 15 minutes in a horizontal shaker. 1 ml of the suspension was again diluted in 9 ml sterile water to get a dilution factor of 10^{-2} g soil per ml suspension. Similarly, 10^{-3}, 10^{-5} and10^{-6} diluted suspensions were prepared for fungal, actinomycetes and bacterial population studies.0.1 ml of 10^{-3}dilution was plated in sterilized Petri dishes containing 20 ml solidified potato dextrose agar (PDA, supplemented with Rose Bengal 33mg/L) medium. The inoculated dishes were incubated at 30±2°C for 4 days.

For isolation of actinomycete, 10^{-5} soil suspensions were inoculated in solidified agar media (Hi-Media) and inoculated plates were incubated at 30±2°C for 2 weeks. For isolation of bacteria, 10^{-6} soil suspensions was inoculated in nutrient agar (Hi-Media) medium by spread plate methodincubated at 30±2°C for 24 hours. The microbial colonies werecounted and expressed in per gram of oven dry soil. The population of fungi, bacteria and actinomycetes were calculated in terms of colony forming units (CFU) per grame oven dry weight basis.

One way analysis of variance (ANOVA) was used to study the variation on distribution pattern of microbial population between sites and seasons respectively.

RESULTS

Soil textures of rhizospheric soilsinthe three medicinal plants, *A. racemosus, C.colebrookianum*and *V. negundo*wassandy loam while in other two plants, *C. teeta* and *T. sinensis*the soil texture was loamy fine sand. Soil temperature ranged from a minimum of 3.4°C in the winter season to a maximum of 24.6°C during summer season (Table 1). Soil moisture content also varied from a minimum of 14.8% during winter season to a maximum of 64.8% during summer season. Soilreactions of all soils were acidic (pH 3.2 to 6.5). Total organic carbon ranged from 1.0 to 6.3% while total nitrogen varied from 0.01 to 0.35%. Total phosphorus ranges from 0.07 to 0.29 % among the different rhizosphere soils.

Fungal population in the rhizosphere of fivemedicinal plants varied from 17x$10^3$to 33x 10^3 CFU/g dry soil (Figure 1A). Highest fungal population was recorded from the rhizosphere of *C. colebrookianum,* followed by *A. racemosus*, *V. negundo* and C. teetain summer seasonswhereas the lowest population was recorded from the rhizosphere soil of *T. sinensis* during winter season. In general, fungal population was high in summer as compared to the winter season in all the rhizosphere soils.One way analysis of variance (ANOVA) revealed significantlyhigher variation (F=68; $p \geq 0.001$) in the population of fungus between seasons as compared to the variation due to plant species (Table 2)

Bacterial population in the rhizosphere soils of medicinal plant varied from 37x10^6 to 63x 10^6 CFU/g dry soil (Figure 1B). Highest bacterial population was recorded from the rhizosphere soil of in *C. teeta* followed by *T. sinensis*, *A. racemosus*during summer seasons. Lowest population of bacteria was recorded from the rhizosphere soil of *C. teeta* during winter season. In general, bacterial population was high during summer seasonas compared to the

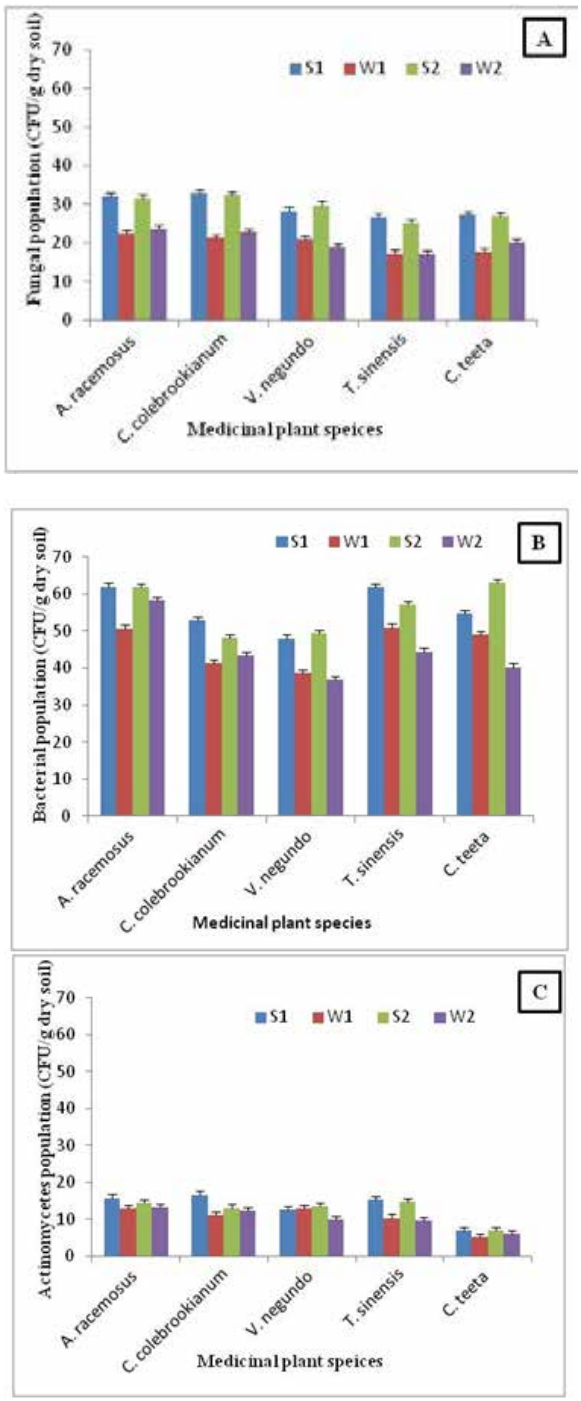


Figure 1 (A-C). Distribution of fungal (A), bacterial (B) and actinomycetes (C) populations in the rhizosphere of five medicinal plants during different seasons. (S1- Summer 1; W1-Winter 1; S2-Summer 2 and W2- Winter 2).

winter season in all the rhizosphere soils. There was significant variation (F=11; $p \geq 0.001$) in the population of bacteria between seasons as well as plant species (F=7; $p \geq 0.01$).

Actinomycetes population was ranged from 5x10^5 to 17x10^5 CFU/g dry soil (Figure 1C). Highest population was recorded in *C. colebrookianum*, followed by *A. racemosus, T. sinensis* in summer and lowest recorded in *C. teeta* in winter. All the studied rhizopshere soil microbes show higher population in summer as compared to winter. There was no significant variation in the actinomycetes population between seasons. However, the distribu-

tion of actinomycetes was found significantly different between the rhizosphere soils of different plant species (F=4; $p \geq 0.05$).

Table 1: Physico-chemical properties of soils in the rhizosphere soil of five selected medicinal plants.

Plant species	Season	SM (%)	ST (°C)	pH	TC (%)	TN (%)	TP (%)
A. racemosus	W	27.2±0.1	17.0±0.2	6.1±0.04	1.7±0.07	0.12±0.006	0.14±0.003
	S	29.9±0.4	21.0±1.9	5.7±0.04	1.8±0.06	0.15±0.005	0.15±0.004
C.colebrookianum	W	15.7±0.2	15.4±1.3	5.9±0.03	1.4±0.02	0.17±0.004	0.10±0.004
	S	21.6±0.3	23.5±1.6	5.6±0.02	2.3±0.05	0.13±0.004	0.09±0.004
V. negundo	W	15.9±0.3	21.0±0.9	6.2±0.02	2.3±0.06	0.23±0.002	0.13±0.002
	S	20.8±0.2	24.1±2.3	5.5±0.03	2.6±0.06	0.17±0.005	0.10±0.003
T. sinensis	W	21.2±0.2	15.9±0.3	6.1±0.04	2.1±0.09	0.23±0.005	0.08±0.003
	S	26.1±0.5	23.4±2.6	5.3±0.04	3.5±0.04	0.34±0.006	0.08±0.002
C. teeta	W	56.8±0.4	3.6±1.2	4.1±0.04	6.2±0.02	0.28±0.005	0.28±0.006
	S	63.9±0.3	10.5±1.0	3.5±0.03	5.9±0.03	0.31±0.006	0.27±0.005

(SM-Soil moisture; ST- Soil temperature; TC- Total organic carbon; TN- Total nitrogen; TP- total phosphorus; Pa- available phosphorus; Mean value ±SD)

DISCUSSION

Seasonal variation of microbial population in rhizosphere soil of medicinal plants

Present study revealed that distribution of fungal population was significantly influenced by seasons as compared to variation due to plant species. These findings are in agreement with other similar works reported from the rhizosphere soil of different medicinal plants. Highest population of fungi have been reported in the rhizospheresoils of different aged mulberry (*Morus spp*.) and *Aeglemarmelos*plants(Chowdary*et al.,* 2014; Shilpkar*et al.*,2010). Similarly, occurrence of lower fungal population was reported from therhizosphere soil grass

Table 2. ANOVA of microbial populations in the rhizosphere soils of five medicinal plants

Organisms	Source of variable	F-value	*p*-value	Remarks
Fungi	Site (plant species)	8	0.00	S
	Time (Season)	67	0.00	S
	Site X Time	0	1.00	NS
Bacteria	Site (plant species)	7	0.01	S
	Time (Season)	11	0.00	S
	Site X Time	0	1.00	NS
Actinomycetes	Site (plant species)	4	0.05	S
	Time (Season)	0	0.98	NS
	Site X Time	0	1.00	NS

species in the Western Ghats of India during summer as compared to winter season (Shivanna and Vasanthakumari, 2011). The reasons for occurrence of maximum population of fungi could be due to favourable microclimatic conditions prevailing in the rhizosphere regions of *C. colebrookinum* and *A. racemosus.*

Bacterial population in the rhizosphere soils of the medicinal plants were also influenced by both seasons as well as plant species. Mahasneh*et al.,* (1984) reported increased bacterial population in the rhizosphere of *Suaeda* and *Arthrocnemum* species during summer seasons. Significant impact of seasons and plant species on soil bacterial communities has been reported by Smalla*et al.,* (2001).Koeberl*et al.,* (2013) also reported significant variation in distribution of microbial population as influenced by different plant species. Their study revealed thatrhizosphere microbes of three medicinal plants *Matricariachamomilla*, *Calendula officinalis* and *Solanumdistichum*were found to be related with a plant-specific selection of the microbes. The seasonal fluctuations of bacterial population are due to climate and soil variables that affect the total number of bacteria in the soil (Rigobelo and Nahas, 2004).The other reasons for higher population of fungi and bacteria during summer season could be due to favorable temperature and moisture contents of the rhizosphere soil which favors rapid multiplication and growth of microbes (Bhattacharyya and Jha, 2011). Microbial population is reported to be lower in winter periods due to low moisture content of soil and temperature thereby inhibiting the microbial activity and growth (Devi and Yadava, 2006).

Actinomicetespopulation in the present study revealed comparatively lower population as compared to fungal and bacterial communities. There was no significant impact of seasons on the distribution of this group of organism. However, there was a profound influence of plant species onactinomycetes population.ActinomycesesBasil *et al.,* (2004) reported occurrence plant specific species of actinomycetes in the therhizosphere of big sagebrush (*Artemisia tridentata*). Gonzalez-Franco *et al.*,(2009) studied on temporal variation of actinomycete communities of the rhizosphere of the desert plant *Artemisia tridentate* (sagebrush) and analysis showed that actinomycete counts was higher in spring. In the present study, it was observed that acinomycetes population was lowest in the rhizosphere soil of *C. teeta*as compared to other medicinal plants.This finding could be related to the microclimatics low temperature conditions of the habitat where this high medicinal plant prefers to grow. However, it has been reported thatactinomycetes are heat and water stress tolerable and profusely reported from desert soils (Zenova*et al.,* 2007).

The population richness of microbes in particular rhizosphere of plant species variable due to influence of root exudates released by the plant roots (Broecklin*get al.,* 2008).Collado*et al.* (1999)and Gao*et al.* (2005) also reported seasonal influence of plant species on distribution of the rhizospheric microorganisms. In the present study bacterial population was comparatively higher than fungi and actinomycetes in all the seasons. This findings are also in conformity with the reports of higher bacterial population followed by fungus and actinomycetesrecorded from the rhizosphere of different medicinal plants (Tamilarasi*et al.*,2008; Karthikeyan, *et al.,* 2008). It was observed that bacterial population showed significantpositive correlation with moisture in both the seasons whereas fungi and actinomycetes did not show positive correlations with moisture contents of the rhizosphere soils.Brockett*et al.* (2012) reported that soil moisture content is a major controlling variable on microbial community structure and enzyme activity in the rhizosphere region of plants.

The present study reveals that distribution of microbial populations (fungal, bacterial and actinomycetes) in the rhizosphere soil of medicinal plants are significantly influenced by variation in seasons as well as different plant species. The effect of time in terms of winter and summer seasons markedly affected the distribution of bacterial population as compared to plant species. Soil moisture was found to be one of the determining soil physical parameter influencing bacterial populations in the present study. Other microclimatic paramaters such as soil temperature, nutrient availability and types of exudates released by plant roots could also play key roles in the distribution of microbial

populations in the rhizosphere soils of medicinal plants.

REFERENCE

1. Basil, A.J., Strap, J.L., Knotek-Smith, H.M. andCrawford, D.L. (2004). Studies on the microbial populations of the rhizosphere of big sagebrush (Artemisia tridentata). J IndMicrobiolBiotechnol, 31(6):278-88. | 2. Batten, K. M., Scow, K. M., Davies, K. F. and Harrison, S. P., (2006). Two invasive plants alter soil microbial community composition in serpentine grasslands. Biological Invasions,8:217–230. | 3. Bhattacharyya, P.N. and Jha, D.K., (2011), Seasonal and depth wise variation in microfungal population numbers in Nameri forest soil, Assam, northeast India. Mycosphere, 2(4): 297-305. | 4. Brimecombe, MJ; FA De Lelj& JM Lynch. 2001. The Rhizosphere. The Effect of Root Exudates on RhizosphereMicrobil Populations. In: R Pinton; Z Varanini& P Nannipieri (eds.). The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface. Marcel Dekker, New York, pp 95-140. | 5. Brockett, B.F.T., Prescott, C.E. and Grayston, S.J., (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry, 44: 9-20. | 6. Broeckling, C. D., Broz, A.K., Bergelson, J., Manter, D. K. and Vivanco, J. M., (2008). Root Exudates Regulate Soil Fungal Community Composition and Diversity. Applied and Environmental Microbiology, 74(3): 738–744. | 7. Chowdary, N. B., Satyanarayana, Ch. R., Thippeswamy, T. and Bindroo, B. B. (2014).Highest population of fungi have been reported in the rhizosphere of different aged mulberry (Morus spp.). Agriculture and Soil Sciences, 1(1): 007-011. | 8. Collado, J., Platas, G., Gonzalez, I., Pelaez, F. (1999). Geographical and seasonal influences on the distribution of fungal endophytes in Quercus ilex. New Phytologist 144: 525–532. | 9. Das, B.B. and Dkhar, M.S., (2011), Rhizosphere microbial populations and phisico chemical properties as affected by organic and inorganic farming practices. American- Eurasian journal of Agricultural Environmental Science, 10(2):140-150. | 10. Devi, N.B. and Yadava, P.S. (2006). Seasonal dynamics in soil microbial biomass C, N and P in a mixed oak forest ecosystem of Manipur, North-east India. Applied Soil Ecology, 31(3): 220–227. | 11. Gao, X.X., Hui, Z., Xu, D.Y., Chen, Y.Q., Qu, L.H. (2005). High diversity of endophytic fungi from pharmaceutical plant Heterosmilax japonica Kunth revealed by cultivation independent approach. FEMS Microbiology Letters, 249, 255–266. | 12. Garbeva, P., Elsas, J. D. van and Veen , J. A. Van, (2008). Rhizosphere microbial community and its response to plant species and soil history. Plant and Soil, 302:19–32. | 13. Girlanda ,M. and Perotto , S., (2005). A century of rhizosphere research: Fungal interections with plant's hidden half. Mycological research, 109: 1058-1061. | 14. Gonzalez-Franco, A.C., Robles-Hernandez, L., Nuñez-Barrios, A., Strap J.L. and Crawford, D.L., (2009). Molecular and cultural analysis of seasonal actinomycetes in soils from Artemisia tridentate habitat, international Journal of Experimental Botany, 78: 83-90. | 15. Innes, L., Hobbs, P. J. and Bardgett, R. D., (2004). The impacts of individual plant species on rhizosphere microbial communities in soils of different fertility. Biology and Fertility of Soils,40:7–13. | 16. Jones, D.L., Hodge, A., and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. New Phytologist, 163: 459-480. | 17. 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: Bioinerfaces, 62:143-145. | 18. Koeberl M, Schmidt R, Ramadan EM, Bauer R, Berg G (2013) The microbiome of medicinal plants: diversity and importance for plant growth, quality, and health. Frontiesr in Microbiology, 4:400. | 19. Mahasneh, A., Budour, S., and Doddema, H., (1984). Nitrification and seasonal changes in bacterial populations in the rhizosphere of Suaeda and Arthrocnemum species growing in saline soils. Plant and soil, 82:149- 154. | 20. Mougel, C., Offre, P., Ranjard, L., Corberand, T., Gamalero, E., Robin, C. and Lemanceau. P., (2006). Dynamic of the genetic structure of bacterial and fungal communities at different developmental stages of MedicagotruncatulaGaertn. cv. Jemalong line J5. New Phytologist, 170:165–175. | 21. Okaleo, J.R., Gathua, K.W. and Woomer, P.L., (1993). Laboratory methods of Soil and Plant Analysis.Aworking manual. TSBF. Nairobi, Kenya. | 22. Rigobelo, E.C. and Nahas, E., (2004). Seasonal fluctuations of bacterial population and microbial activity in soils cultivated with eucalyptus and pinus. Scientia Agricola, 61(1). | 23. Shilpkar P., Shah, M. C., Modi K. R., Patel S. M. (2010). Seasonal changes in microbial community structure and nutrients content in rhizospheric soil of Aeglemarme Los tree. Annales for Forest Research, 53(2): 135-140. | 24. Shivanna, M.B. and Vasanthakumari, M.M. (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. | 25. Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H. and Berg, G., (2001).Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-Dependent Enrichment and Seasonal Shifts Revealed. Applied and Environmental Microbiology, 67(10): 4742- 4751. | 26. Tamilarasi, S., Nanthakumar, K., Karthikeyan, K. and Lakshmanaperumalsamy, P. (2008). Diversity of root associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of Coriandrumsativum. Journal of Environmental Biology, 29(1): 127-134. | 27. Yang, Q. Wang, X. and Shen, Y. (2013). Comparison of soil microbial community catabolic diversity between rhizosphere and bulk soil induced by tillage or residue retention. Journal of soil science and plant nutrition, 13(1). | 28. Zenova, G.M., Gryadunova, A.A., Doroshenko, E.A., Likhacheva, A.A., Sunnitsyn II, Pochatkova, T.N. and Zvyagintsev, V. (2007). Influence of moisture on the vital activity of actinomycetes in a cultivated low-moor peat soil. Eurasian Journal of Soil Science, 40: 560-564.