AATCC Review

13 November 2022: Received
19 March 2023: Revised
27 May 2023: Accepted
07 July 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Dynamics of Microbial Communities in Bamboo Rhizosphere Soil under Natural Ecosystem

Vennila.S[1]*, T.Kalaiselvi[2], C. Cinthia Fernandaz[3] and K. Sivagamy[4]

[1]Agricultural College and Research Institute, Tiruvannamalai, India
[2]Tamil Nadu Agricultural University, Coimbatore, India
[3]ICAR- Krishi Vigyan Kendra, Nilgiris, India
[4]ICAR- Krishi Vigyan Kendra, Tiruvallur, Tirur, India

ABSTRACT

The rhizosphere soil microbial dynamics and fertility status of the bamboo in different locations viz., Anaikatty, Barliyar, Mammaram, Gudalur, and Mettupalayam were studied. In general, the density of microflora varied widely among locations as well as between bamboo rhizosphere and non-rhizosphere soils. The density of bacteria and actinomycetes was found to be higher in Anaikatty rhizosphere soil and the fungal population was higher in Mettupalayam non-rhizosphere soil. Among the bamboo rhizosphere, Anaikatty harbored a greater number of microbial populations while Barliyar rhizosphere soils exhibited greater microbial diversity. Comparing rhizosphere and non-rhizosphere soils, non-rhizosphere were microbially more diverse. Among natural and managed ecosystems, natural ecosystems had more number of bacteria and actinomycetes, while managed ecosystems recorded a greater number of fungi. Pseudomonas and Streptomyces were the dominant bacterial and actinomycetes genera encountered in the study area. The mycorrhizal infection and AM spore population were greater for managed ecosystems over the natural ecosystems.

Keywords: *Rhizosphere, non-rhizosphere, bamboo, microbes, ecosystems*

INTRODUCTION

Microorganisms constitute less than 0.5 percent (w/w) of the soil mass, yet they have a major impact on soil properties and processes. The seemingly rigid mass of clay, sand, and silt is home for a complex microbial community including bacteria, fungi, actinomycetes, algae, protozoa, and viruses. The soil bacteria and fungi play pivotal roles in various biogeochemical cycles (BGC) [20] and are responsible for the cycling of organic compounds. Soil microorganisms also influence above-ground ecosystems by contributing to plant growth and soil fertility [21]. Thus, the intimate association of microbes and soil particulates is critical for total ecosystem survival. Hence, the integrity of the total ecosystem, above and below ground, rests on the stability of the soil microbial community. The destruction of the soil microbiota through mismanagement or environmental interference results in the decline or even death of the above-ground plant population. Thus, an understanding of soil microbes, their properties and the nature of their interaction with and within their environment is essential. With this background, the study on the assessment of the rhizosphere microbial community of bamboo was undertaken.

**Corresponding Author: Vennila.S*
Email Address: vennila.s@tnau.ac.in

DOI: https://doi.org/10.58321/AATCCReview.2023.11.03.335

MATERIAL AND METHODS

Bamboo rhizosphere soil samples were collected from different locations *viz*., Anaikatty, Barliyar, Mammaram, Gudalur, and Mettupalayam. Among these locations, Mettupalayam plantation was artificially raised whereas the other locations were natural strands. For comparison, non-rhizosphere soil samples adjacent to bamboo plantations were also collected and analyzed for the microbial population. The soil samples were packed in sterile polythene bags and brought to the laboratory for further analysis. The soil samples were stored at 4°C till they were processed for the following dynamics of microbes.

The dynamics of bacteria, fungi, actinomycetes, diazotrophic bacteria, and phosphate solubilizers were enumerated using serial dilution and plating techniques [10].

Serial dilution of the sample

One gram fresh soil was transferred to 100 ml sterile distilled water to get 10^{-2} dilution. After thorough shaking, 1 ml of the dilution was transferred to 9 ml water blank to get 10^{-3} dilution. Likewise, the samples were diluted serially with 9 ml water blanks until appropriate dilution was obtained.

The bacteria was enumerated by plating one ml of 10^{-7} dilution in the sterile petri dishes using a Nutrient Agar medium. The colonies appearing on the plate after 48 hours of incubation at 30°C were counted and expressed as a number of CFU.g^{-1} soil.

Fungi were enumerated by plating one ml of 10^{-6} dilution in the sterile petri dishes using Martin's Rose Bengal Agar medium. The colonies appearing on the plate after 2-3 days of incubation were counted and expressed as the number of CFU. g^{-1} soil.

One ml of 10^{-2} dilution was transferred to sterile petri dishes and plated in Kenknight's Agar medium and incubated. The colonies of actinomycetes that appeared after 10-14 days were counted and expressed as the number of CFU. g^{-1} soil.

Isolation of diazotrophic microorganisms

The dinitrogen-utilizing microbes such as *Azotobacter, Azospirillium,* and *Beijerinckia* were isolated from bamboo rhizosphere soil samples collected from various locations *viz.,* Anaikatty, Barliyar, Mammaram, Gudalur, and Mettupalayam.

One ml of 10^{-4} dilution was transferred to sterile Petri dishes and plated in Waksmann No 77 medium and incubated. The colonies of *Azotobacter* that appeared after 4-5 days were counted and expressed as the number of CFU.g^{-1} soil.

One ml of 10^{-4} dilution was transferred to sterile Petri dishes and plated in nitrogen-free malic acid medium and incubated at 30°C for 3-4 days. The colonies of associative nitrogen-fixing bacterium Azospirillum were counted after change of medium colour from yellowish green to brilliant blue.

For enumeration of *Beijerinckia*, one ml of 10^{-5} dilution was transferred to sterile petri dishes and plated in *Beijerinckia* medium and incubated. The colonies of *Beijerinckia* that appeared after 4-5 days were counted and expressed as a number of CFU.g^{-1} soil.

Bacteria that solubilize the unavailable form of phosphates, known as phosphate solubilizing bacteria were enumerated using Sperber's hydroxy apatite medium. One ml of 10^{-3} dilution of the soil samples was transferred to sterile Petri dishes and added with 20 ml of Sperber's hydroxy apatite medium. The bacterial colonies with well-developed clear zone around them were enumerated after five days and expressed as the number of CFU.g^{-1} soil.

Estimation of AM fungal spores in soil

Vesicular arbuscular mycorrhizal (AM) fungal spores were isolated from rhizosphere and non-rhizosphere soil by wet sieving and decantation technique [5].

Soil samples (100 g) were taken in a one-litre beaker and water (1000 ml) was added and stirred well and kept undisturbed for 1 min. The aqueous portion was passed into five sets of sieves of 1 mm, 450 m, 300 m, 250 m, 105 m, 53 m and 25 m. Residues from 450 m, 300 m, 250 m, and 105 m sieves were collected and pooled and the volume was made upto 100 ml. Spore count was taken, in every 2 ml of the suspension, using a binocular microscope. The spore number was expressed as the number of spores per gram of soil.

Examination of AM fungal infection in bamboo roots

The AM colonization was estimated by adopting the procedure described by Phillips and Hayman [12].

Plants roots were collected and washed carefully to remove the adhering soil particles. The roots were cut into approximately 2 cm segments. The root bits were immersed in 10 per cent potassium hydroxide and autoclaved for 15 min. at 10 lbs pressure. After this, the potassium hydroxide was decanted and immersed in two per cent hydrochloric acid for 15 min to neutralize the excess potassium hydroxide present. The root bits were then immersed in 30 percent hydrogen peroxide solution for 15-30min. The hydrogen peroxide solution was decanted and the root bits were rinsed with water and then washed with tap water and stained with 0.05 percent trypan blue in lactic acid; glycerol; distilled water (1:2:2 v/v) for 24 hours. The excess stain was removed by treating the root pieces with lactophenol. Mycorrhizal infection in the root pieces was observed using a binocular microscope (10 x).The percent mycorrhizal colonization was then calculated as,

$$\text{Per cent infection} = \frac{\text{Number of positive segments}}{\text{Total number of root segments observed}} \times 100$$

RESULT AND DISCUSSION

The rhizosphere is most certainly an area of intense biological activity within the soil ecosystem. It is represented by the dynamics of microbial population, the complete range of plant-microbe and microbe–microbe interactions and the relative inclusiveness of all essential biogeochemical processes for total ecosystem development. Hence, the productivity of the microbial community and the above-ground community is interlocked with the viability and stability of the microbial community. With this in view, the results of the study are discussed here under.

In general, bacterial population was greater in rhizosphere soil compared to nearby non-rhizosphere. Anaikatty rhizosphere soils harbored more number of bacteria (585.75 x 10^7 CFU. g^{-1} soil) than other locations. On the contrary, the non-rhizosphere soils of Anaikatty recorded the lowest bacterial population of 2.00 x 10^7 CFU. g^{-1} soil. Of the rhizosphere soil samples, lowest bacterial population of 33.25 x 10^7 CFU. g^{-1} soil was recorded in the soil samples of Mettupalayam. The statistically significant difference in rhizosphere bacterial population was observed between Anaikatty and other locations. But no differences in bacterial population were observed between other locations (Table 4). Comparing the natural ecosystem with managed ecosystem, managed ecosystem recorded least number of microbes. The qualitative analysis indicated that the pseudomonds population was greater in all locations (Table 2). The qualitative analysis of the microbial population revealed the presence of greater number of Gram-negative bacteria, *Pseudomonas* in all locations except Mettupalayam and Mammaram where in more number of B*acillus* was encountered. In addition, many antagonistic bacteria were isolated from different locations except Mammaram (Table 2).

In contrast to the bacterial population, the highest fungal population was recorded in the Mettupalayam non-rhizosphere soil (43.75 x 10^6 CFU. g^{-1} soil). Among the locations, the maximum population was registered in the rhizosphere and non-rhizosphere soils of Mettupalayam. Even though, statistically significant difference was noticed between Mettupalayam and other locations of non-rhizosphere, no significant difference was observed among the locations of rhizosphere soils (Table 4). Among managed and natural ecosystems, the managed ecosystems had more fungi. The qualitative analysis recorded that *Fusarium* was dominant in all locations except Gudalur non-rhizosphere soil (Table 3). Apart from *Fusarium, Penicillium, Rhizopus* and *Trichoderma* were observed in different study area. The Mettupalayam soils had more *Penicillium*.

Similar to bacteria, the rhizosphere soil samples of Anaikatty recorded the highest actinomycetes population (1999.5 x 10^2 CFU. g^{-1} soil). The minimum population of 139.88 x 10^2 CFU. g^{-1} soil was registered in the rhizosphere soil samples of Gudalur. Among non-rhizosphere soil samples, the highest population of 16.75 x 10^2 CFU. g^{-1} soil was obtained from soil samples of Barliyar. Similar to bacteria, the results of non-rhizosphere soil samples were found to be statistically nonsignificant.

No significant difference in the actinomycetes population was noticed among locations. In case of rhizosphere soil samples, a significant difference was observed between Anaikatty and other locations (Table 3). With regard to actinomycetes, the natural ecosystem recorded higher number than the managed ecosystem and Streptomyces was the dominant flora in all locations.

In general, the density of bacteria and actinomycetes was higher in rhizosphere soils. Among the locations, Anaikatty rhizosphere soils recorded maximum population of bacteria and actinomycetes the least in bamboo rhizosphere soils of Mettupalayam. On the contrary, maximum fungal population was noticed in nonrhizosphere soil samples of Mettupalayam. The soils of Gudalur registered for the lowest population of actinomycetes owing to their acidic pH [1]. In general, the actinomycetes population was found to be the least among the microbes analysed. This may be due to cool temperatures and acidic environments prevalent in various locations except Anaikatty and Mettupalayam (Table 1). Similarly, the wide variation in bacterial and actinomycetes population among locations and between rhizosphere and nonrhizosphere soils may be due to varied physicochemical properties of the soils *viz*., pH, organic carbon and available nutrients. This is in accordance with the report that changes in average number of fungi and bacteria is Kumaun Himalaya soils are positively correlated with soil moisture and negatively with soil pH [18]. The highest available nitrogen and organic matter content of the Anaikatty soils may be responsible for greater bacterial and actinomycetes observed in the study area.

The qualitative analysis of fungi revealed the occurrence of various fungal species like *Fusarium, Penicillium, Rhizopus,* and *Trichoderma.* Among these, *Fusarium* was the dominant genus in all locations followed by *Penicillium* and *Rhizopus. Trichoderma* was obtained from soils of Mettupalayam only. Similar studies observed species composition, population and biomass of microfungi in tropical forest soil of Orissa and reported that *Aspergillus* and *Trichoderma viride* were the dominant genera among fungi imperfect [3]. The dominance of these fungi over other genera may be due to their high sporulating ability and rapid growth. The *Penicillium* is recorded as the dominant genus in the soils of the Nilgiris high altitude forest [16]. The results of the present study are agreement with the above observation. Apart from these, *Monascus,* a red pigment-producing yeast was obtained from the rhizosphere soil samples of Gudalur. In case of actinomycetes, *Streptomyces* was the dominant flora in all locations. The dominance of *Streptomyces* species among the soil actinomycetes in all types of soil, including desert soil [4] [17] was reported by several workers [2] [9]. In addition, unidentified red-pigmented actinomycetes too were encountered in the soils of Mammaram and Gudalur.

Dynamics of dinitrogen fixers

The *Azotobacter* population in the rhizosphere and non-rhizosphere soils of different locations ranged between 0.50 x 10^4 CFU. g^{-1} soil and 17.50 x 10^4 CFU. g^{-1} soil. However, the rhizosphere soils of Anaikatty registered higher values (17.50 x 10^4 CFU. g^{-1} soil) compared to other locations. An *Azotobacter* isolate capable of solubilizing dicalcium phosphate was also isolated from Anaikatty rhizosphere soils. The lowest *Azotobacter* population was noticed in the Mammaram non-rhizosphere soil (0.50 x10^4CFU. g^{-1} soil). Statistically significant variation in the *Azotobacter* population was observed between bamboo rhizosphere and non-rhizosphere soil samples (Table 5). In general, natural ecosystems recorded more *Azotobacter* than managed ecosystem.

The highest *Azospirillum* population was recorded in Gudalur rhizosphere soil (24 x 10^4 CFU. g^{-1} soil). This was followed by Mettupalayam rhizosphere soil 20.25 x 10^4 CFU. g^{-1} soil). The lowest *Azospirillum* population was observed in the Gudalur non-rhizosphere soil (1 x 10^4 CFU. g^{-1} soil). Statistically no significant difference was observed in the *Azospirillum* population between the non-rhizosphere soil samples (Table 5). In contrast to *Azotobacter,* managed ecosystem had more number of *Azospirillum.*

Similar to bacteria and actinomycetes, the rhizosphere soil samples of Anaikatty recorded the highest *Beijerinckia* population (32.50 x 10^5 CFU. g^{-1} soil). The minimum population of 5.40 x 10^5 CFU. g^{-1} soil was recorded in the soil samples of Mettupalayam. Among the non-rhizosphere soil samples, the highest population of 1.58 x 10^5 CFU. g^{-1} soil was obtained from soil samples of Anaikatty (Table 5). Among natural and managed ecosystems, natural ecosystems harbored more number of *Beijerinckia*. In case of rhizosphere soil samples, significant difference was observed between Anaikatty and other locations. Similar to actinomycetes, the results of non-rhizosphere soil samples were found to be statistically nonsignificant.

Anaikatty soils harbored greater number of *Azotobacter*. This may be due to higher organic matter content of the Anaikatty soils. While the maximum *Azospirillum* population was observed in the soils of Gudalur. Even though, these organisms are neutral pH-preferring microbes, a good number of *Azospirillum* isolates were obtained from Gudalur soils whose pH is 4.41. Hence, these isolates may be of acid-tolerant species. Further studies are needed to confirm the results. Similar to the present study, the occurrence of *Azotobacter, Azospirillum,* and *Beijerinckia* in various tropical forests has been reported by many workers [11] [13] [14] [15] [19].

Phosphorus dynamics and solubilizing microorganisms

The phosphate solubilizing microbial population *Pseudomonas, Fusarium* and *Aspergillus* in rhizosphere and non-rhizosphere soils of different locations ranged between 0.5 x 10^3 CFU. g^{-1} soil and 7.25 x 10^3 CFU. g^{-1} soil. Anaikatty rhizosphere soils harbored the highest population of phosphate solubilizing microbes (7.25 x 10^3 CFU. g^{-1} soil) followed by Barliyar bamboo rhizosphere soil (5.50 x 10^3 CFU. g^{-1} soil) (Table 6). There was statistically no significant difference in the density of phosphate solubilizing microorganisms between the non-rhizosphere soils. However, statistical analysis revealed that there is significant difference among rhizosphere bacterial populations. It is also noticed that there is no significant difference between natural and managed ecosystems.

Insoluble inorganic compounds of phosphorus are largely unavailable to plants, but many microbes bring the phosphate into solution. Hence, these microbes dominate in fertile soils that is, soils low in available phosphorus. In the present investigation, maximum phosphate solubilizing microbes were obtained from the soils of Anaikatty whose available phosphorus content is the least among the locations studied.

A minimum number of isolates was observed in the soils of Gudalur whose available phosphorus content is greater. It may be due to low insoluble phosphate content of the soils. Since the phosphate solubilizing microbes activity is generally greater in soils with greater fixed form of phosphates [8]. It was also observed that there were more number of fungal isolates than

phosphate-solubilizing bacteria. Among the bacterial and fungal isolates, the phosphate solubilizing ability of fungi was found to be greater. Based on morphological and cytological observation, the bacterial isolates were found to be *Pseudomonads* and fugal isolates were *Fusarium* and *Aspergillus*. Further, the phosphate solubilizing ability of these microbes was evaluated qualitatively by the formation of halo zone around colonies growing on Sperber's hydroxy apatite medium. The results of the study support that the phosphate solubilizing ability of the fungal isolates from tropical forests (Indonesia) are greater than bacterial strains [6].

AM Spore population and AM infection

The AM spore population ranged between 24 spores / 10-gram soil and 60 spores / 10 gram soil (Table 7). Mettupalayam rhizosphere soils scored higher AM spores of 60 and AM infection of 70%, while Anaikatty soil samples recorded the lowest AM spore population (Table 7). A statistically significant difference in spore population and AM infection was observed between locations. *Glomus* was found to be the dominant genus in all locations.

Another important group of microbes involved in phosphorus nutrition is AM fungi. Unlike nitrate, phosphorus is an immobile element. It is taken up by the plant system only through the diffusion process. In order to improve the phosphorus uptake by the plant system, plants posses the fungal root symbiont called mycorrhizae. Hence, they are more pronounced in less fertile soils. In the present study, maximum mycorrhizal infection and spore count was observed in the Mettupalayam soils. Even though available phosphorus content was less in both Anaikatty and Mettupalayam soils, Mettupalayam soils had more AM spores due to the external application of AM (managed ecosystem) compared to Anaikatty (natural ecosystem) soils.

The AM fungal infection was greater in the root samples of Mettupalayam over other locations. Similarly, a number of spores g^{-1} soil was too found to be higher in the rhizosphere soil samples of Mettupalayam. Highly fertile soils generally show less AM fungal population [7]. So this, may account for a lower number of AM spores and mycorrhizal infection in the natural ecosystem over the managed ecosystems. The results of the present study may even be due to lack or insufficient number of viable spores.

Comparison of microbial load of a natural ecosystem with managed ecosystem exhibited wide variation. In the case of bacteria and actinomycetes, managed ecosystem recorded the least, while the fungal population was greater in managed ecosystem.

Table 1. Physicochemical properties of the bamboo rhizosphere and adjacent non-rhizosphere soil

Location	pH		EC		Organic carbon (%)	
	R	S	R	S	R	S
Anaikatty	6.00^{d}	7.02^{b}	1.898^{a}	0.140^{c}	1.90^{a}	0.33^{b}
Barliyar	6.99^{b}	5.55^{c}	0.837^{b}	0.117^{d}	1.20^{d}	0.70^{a}
Mammaram	6.71^{c}	3.95^{d}	0.232^{d}	0.409^{a}	1.70^{c}	0.70^{a}
Gudalur	4.41^{e}	3.06^{e}	0.457^{c}	0.215^{b}	1.83^{b}	0.73^{a}
Mettupalayam	7.70^{a}	8.00^{a}	0.116^{e}	0.102^{e}	0.73^{c}	0.67^{a}
Mean	6.36	5.52	0.708	0.197	1.47	0.63

Table 2 .The qualitative and quantitative bacterial isolates of various locations

Location	*Bacillus* (X x10^{7} CFU. g^{-1} soil)		*Pseudomonas* (X x 10^{7} CFU. g^{-1} soil)		Antagonistic microbes (X x 10^{7} CFU. g^{-1} soil)	
	R	S	R	S	R	S
Anaikatty	35.0	-	520.0	1.5	1.0	-
Barliyar	17.0	3.0	47.5	1.0	2.5	-
Mammaram	10.0	15.0	30.0	-	-	-
Gudalur	5.0	1.0	84.0	2.0	6.0	-
Mettupalayam	2.0	-	30.0	4.0	1.0	-

Table 3. The qualitative and quantitative fungal isolates of various locations

Location	*Fusarium* (X x 10^{6} CFU. g^{-1} soil)		*Pencillium* (X x 10^{6} CFU. g^{-1} soil)		*Rhizophus* (X x 10^{6} CFU. g^{-1} soil)	
	R	S	R	S	R	S
Anaikatty	7.00	16.50	1.00	-	4.00	1.00
Barliyar	8.00	4.00	2.00	0.50	1.00	-
Mammaram	6.00	9.00	-	-	-	-
Gudalur	8.00	2.00	1.00	-	3.00	2.00
Mettupalayam	2.00	39.00	14.50	-	3.50	4.00

Table 4. Microflora of bamboo rhizosphere and non-rhizosphere soils

Location	Bacteria (X x 10^7 CFU. g^{-1} soil)		Fungi (X x 10^6 CFU. g^{-1} soil)		Actinomycetes (X x 10^2 CFU. g^{-1} soil)	
	R	S	R	S	R	S
Anaikatty	585.75^a	2.00^a	15.50^a	19.50^b	1999.50^a	11.25^a
Barliyar	66.50^b	3.43^a	13.00^a	5.25^b	827.13^{ab}	16.75^a
Mammaram	73.75^b	15.50^a	11.75^a	10.00^b	377.63^b	6.25^a
Gudalur	122.25^b	4.50^a	15.25^a	6.75^b	139.88^b	3.25^a
Mettupalayam	33.25^b	4.75^a	20.75^a	43.75^a	170.50^b	8.00^a
Mean	176.30	6.04	15.25	17.05	702.93	9.10

Table 5. Diazotrophic bacterial dynamics of bamboo rhizosphere and non-rhizosphere soils

Location	*Azotobacter* (X x 10^4 CFU. g^{-1} soil)		*Azospirillum* (X x 10^4 CFU. g^{-1} soil)		*Beijerinckia* (X x 10^5 CFU. g^{-1} soil)	
	R	S	R	S	R	S
Anaikatty	17.50^a	7.13^a	15.50^b	6.75^a	32.50^a	1.58^a
Barliyar	9.25^b	2.25^{ab}	4.75^c	5.25^a	13.50^b	0.35^a
Mammaram	4.00^{bc}	0.50^b	4.75^c	1.25^a	14.75^b	0.20^a
Gudalur	1.25^c	1.00^b	24.00^a	1.00^a	13.50^b	0.88^a
Mettupalayam	4.75^{bc}	2.75^{ab}	20.25^{ab}	4.50^a	5.40^b	0.13^a
Mean	7.35	2.73	13.85	3.75	15.93	0.63

Table 6. The phosphate solubilizing microbial dynamics of bamboo rhizosphere and non-rhizosphere soil

Location	Phosphate solubilizing microbes (X x 10^3 CFU. g^{-1} soil)	
	R	S
Anaikatty	7.25^a	1.25^a
Barliyar	5.50^{ab}	3.00^a
Mammaram	3.75^{bc}	1.75^a
Gudalur	1.50^c	0.50^a
Mettupalayam	3.00^{bc}	3.63^a
Mean	4.20	2.03

Table 7. Occurrence of AM spores in the rhizosphere soil of bamboo and degree of AM infection of bamboo roots

Location	No of spores /10g soil	AM infection (in percent)
Anaikatty	24^c	20^d
Barliyar	28^c	40^b
Mammaram	35^b	50^b
Gudalur	32^b	30^c
Mettupalayam	60^a	70^a
Mean	35.8	42

In column, values followed by a common letter are not significantly different at the 5% level by DMRT

References

1. Alexander, M (1978) Introduction of soil microbiology, (ed.2). Wiley Eastern Limited, New Delhi.
2. Balasundaran, M. (1992) Studies on the actinomyctes from the rhizosphere soils of some crop plants. Doctoral Thesis, University of Kerala, Thiruvananthapuram.
3. Behera N, D P Pati and S Basu (1991) Ecological studies of soil microfungi in a tropical forest soil of Orissa, India. Tropical Ecology, 32(1): 136.
4. Elwan SH and A Diab (1976) Actinomycetes of an Arabian desert soil. Egyptian Journal Botany 19: 111-114.
5. Gerdermann J W and T H Nicolson (1963) Spores of mycorrhizal endogene species extracted from soil by wet sieving and decanting. Transaction of the British Mycological Society, 46: 235-244.
6. Goenadi DH, RA Pararibu, H Isroi, Hartono and R Misman (1999) Phosphate solubilizing fungi isolated from tropical forest soils. Menara Perkebunan, 67(1): 40-51.
7. Hayman DS (1982) Practical aspects of vesicular-arbuscular mycorrhizal. In: Advances in Agricultural Microbiology Ed. NS Subba Rao Oxford & IBH Publishing Co Pvt Ltd, New Delhi. pp. 325-373.

8. Khan AA, Jilani G, Akhtar MS, Naqvi SMS, Rasheed M. (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Journal of Agricultural and Biological Science 1(1):48–58.

9. Lechevalier, H A and M P Lechevalier (1981) Introduction to the order Actinomycetales. In: HP Starr, H Stolp, H G Truper, A Balows and H G Schegel (eds.). The Prokaryotes. A Handbook on Habitats, isolation and identification of bacteria. Vol.II. Springer-Verlag, Berlin. Pp. 1915-1922.

10. Parkinson, D., J.R.G. Gray and S.T. Williams. (1971). Methods for studying the ecology of soil microorganisms. Oxford, Blackwell Scientific Publication. Pp. 116.

11. Patil, S V, Mohite, B V Patil, C D Koli, S H Borase, H P and Patil, V S (2020) "Azotobacter," in Beneficial Microbes in Agro-Ecology, ed. Academic Press (Berlin: Springer), 397–426.

12. Phillips JA and D S Haymann (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhiza fungi for rapid assessment of infection. Mycology Society 55: 158-161.

13. Pii Y, Mimmo T, Tomasi N, Terzano R, Cesco S, Crecchio C (2015) Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review of Biology and Fertility of Soils 51, 403–415.

14. Praneetha Paul (1999) Soil biota in sandal seedling establishment M.Sc. Thesis, Forest College and Research Institute Mettupalayam.

15. Ranjana N, and R Nagaraj (1989) Occurrence of Azotobacter and Beijercinckia in forest soils of Maharashtra. Indian Journal of Forestry 12(2): 112-116.

16. Reddy, TKR (1962) Role of plant cover in distribution of fungi in Nilgiri forest soils. Proceedings of Indian Academy of Science (Sect. B), 56: 185-194.

17. Sun X X, Zhang X, Guo D, Wang H Chu (2015) Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biology and Biochemistry. 88 , 9-18.

18. Sushma-Shail R C, Dubev S, Shail SC, Sati J, Saxena and RC Rubey (1997) Seasonal changes in microbial community in relation to edaphic factors in two forest soils of Kumaun Himalaya. Himalayan Microbial Diversity, 2: 381-391.

19. Venkatachalam, S (2003) Assessment of microbial diversity and fertility status of shola soils of Nilgiris. MSc Thesis, Forest College and Research Institute, Mettupalayam. 152.

20. Wall D H and R A Virginia (1999) Controls on soil biodiversity: insights from extreme environments. Applied Soil Ecology, 13: 137-150.

21. Yao H Z He, MJ Wilson and C D Campbell (2000) Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology, 40: 223-237.