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Rhizosphere Microflora of selected shola tree species

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Abstract
The presence of abundant microorganisms in Shola soil has been observed, and studies indicate their positive impact on the growth of regenerating seedlings. This highlights the potential of microbial inoculants to enhance the development of tree seedlings, despite the absence of specific bio inoculants for Shola tree species. Unfortunately, there is a lack of comprehensive information regarding the microbial communities associated with Shola soils and tree species. Therefore, conducting assessments, Documentation, development, and screening of bio inoculants tailored to Shola species will greatly contribute to the successful establishment and management of Shola tree seeds and seedlings in nurseries. By conducting detailed studies and validating the existing information on microbial communities in specific locations, we can gain a better understanding of the challenges related to the establishment of beneficial microbes such as Rhizobium, Azospirillum, Azotobacter, Frankia, and VAM. Consequently, it can be presumed that microbial factors likely play a significant role in inducing germination and facilitating the establishment of Shola trees words.

Keywords: Shola, microbes, rhizosphere, inoculum, wildlings

1. Introduction
A characteristic feature of Western Ghats region is the presence of the much renowned Shola forests mostly found at an altitude beyond 1700 MSL. These forests broadly belong to the category of Tropical Montane forest and to be specific the Sholas are designated as Southern Montane Wet Temperate Forest (Champion and Seth, 1968).
Common in the Nilgiris, Palani hills and Anamalai hills of Tamil Nadu and parts of Palakkad, Idukki and Wayanad of Kerala, these forests are characterized by a unique climate which is more of a temperate nature, though these forests are found in tropical regions.
Various factors like birds, insects and even larger mammals enhance the process of regeneration of Shola species. In spite of this, the survival and establishment of the seedlings under natural conditions are poor because of the dense canopy. Hence attempts have been made by planting wildlings, though they also have been found to be otiose. To overcome this problem, seedlings can be raised in the nursery through seeds. Since Sholas consist of non – regenerating and slow growing species, various innovative methods have been tried to improve the Shola nursery growth over the past several years.
Shola soil is rich in micro flora and studies have shown that these microorganisms have a beneficial effect on the regenerating seedlings. This throws light on the fact that microbial inoculants can also improve tree seedlings, although there are no specific bio inoculants for shola tree species. Unfortunately, there is a paucity of information regarding the microbes associated with Shola soils and tree species. Therefore, the assessment, documentation, development and screening of bio inoculants specific to shola species will contribute greatly to the establishment and management of shola tree seeds and seedlings in the nursery. Substantiating the available information on microbial communities in particular locations through detailed studies will help in understanding the establishment problems of inoculated beneficial microbes such as *Rhizobium, Azospirillum, Azotobacter, Frankia,* VAM etc.
Hence it may be presumed that some factor of microbial origin might definitely be involved in inducing germination and establishment of sholas.

2. Materials and Methods
2.1 Materials
A laboratory experiment was undertaken at Forest College & Research Institute, Mettupalayam to develop a suitable microbial inoculant for the development of shola wildlings.

The species selected for the study were *Pygeum gardneri* & *Syzygium arnottianum* from the Bandi Shola nursery. Soil samples from the rhizosphere and non-rhizosphere were collected and analyzed for the microbial properties.
For collecting non-rhizosphere soil samples, three sites were selected randomly and samples were drawn from a depth of 0 - 20 cm after removing the top layer of the litter.

2.2 Parameters analysed
Total Bacterial, Fungal and actinomycetes Azospirillum Azotobacter Phosphate solubilizing microorganisms VAM spore population VAM fungal infection in root system of shola tree species in rhizosphere and non- rhizosphere soil samples

2.3 Enumeration of microbes
The enumeration of microbes was carried out on solid media using the serial dilution and plating technique of Parkinson *et al*., (1971) [20] using standard media.

2.4 Enumeration of VAM spore population
Mycorrhizal spore population in soil was determined by using the method of Rhodes and Gerdemann (1975) [18].

2.5 Per cent VAM infection
The extent of VAM infection was estimated by following the method of Philips and Hayman (1970).

3. Results
The quality and quantity of microbial community changes with the growing season of the plant, kind of plants and locations. Hence, microbial habitats are produced continually through root growth. With this in view, microbes associated with rhizosphere in different shola species were studied. The microbes involved in biogeochemical cycling such as nitrogen fixation, phosphate solubilization and phosphate mobilization were isolated from rhizosphere and developed as bioinoculants. Finally, the impact of these specific inoculants on growth and development of shola seedlings was assessed under nursery conditions. The results of these studies are given below.

3.1 Microflora of rhizosphere and non-rhizosphere soil of shola species
The soil samples of rhizosphere and nearby non-rhizosphere of different shola tree species *viz*., *Pygeum gardneri, Syzygium arnottianum* were collected and analysed for bacterial, fungal and actinomycetes population.

3.2 Bacterial population
In general, bacterial population was greater in rhizosphere soil compared to nearby non-rhizosphere. *Pygeum gardneri* rhizosphere soils harboured more number of bacteria 14.625 x 10^7 CFU. g^{-1} soil) (Table 1). On the contrary, rhizosphere soils of *Syzygium arnottianum* recorded the lowest bacterial population of 8.8 x 10^7 CFU. g^{-1} soil. Of the non rhizosphere soil samples, lowest bacterial population of 1.5 x 10^7 CFU. g^{-1} soil was recorded in the soil samples of *Pygeum gardneri* (Table 1). Comparing natural ecosystem with managed ecosystem, managed ecosystem recorded least number of microbes. The qualitative analysis indicated that pseudomonas population was greater in all locations. The qualitative analysis of microbial population revealed the presence of greater number of gram negative bacteria, *Pseudomonas*. In addition, many antagonistic bacteria were isolated from different locations (Table 1)

3.3 Fungal population
In contrast to bacterial population, the highest fungal population was recorded in the non-rhizosphere soil (3.5 x 10^6 CFU. g^{-1} soil) (Table 2). Among the locations, maximum population was registered in the rhizosphere and non-rhizosphere soils of *Pygeum gardneri.* The qualitative analysis recorded that *Fusarium* was dominant in all shola tree species Apart from *Fusarium, Penicillium, Rhizopus* and *Trichoderma* were observed in different study area. The rhizosphere soils had more number of *Penicillium.*

3.4 Actinomycetes
Similar to bacteria, the rhizosphere soil samples of *Syzygium arnottianum* recorded the highest actinomycetes population (8 x 10^6 CFU. g^{-1} soil) (Table 3). The minimum population of 7 x 10^6 CFU. g^{-1} soil was registered in the rhizosphere soil samples of *Pygeum gardneri.* Among non-rhizosphere soil samples, the highest population of 16.5 x 10^6 CFU. g^{-1} soil was obtained from soil samples of *Pygeum gardneri.*

3.5 *Azotobacter*
The *Azotobacter* population in rhizosphere and non-rhizosphere soils of different shola tree species between 17.0 x 10^4 CFU. g^{-1} soil and 9.5 x 10^4 CFU. g^{-1} soil (Table 4). However, rhizosphere soils of *Pygeum gardneri* registered higher value (17.0 x 10^4 CFU. g^{-1} soil) (Table 4) compared to other tree species.

3.6 *Azospirillum*
The highest *Azospirillum* population was recorded in *Pygeum gardneri* rhizosphere soil (15.5 x 10^4 CFU. g^{-1} soil). This was followed by rhizosphere soil of *Syzygium arnottianum* 9.5 x 10^4 CFU. g^{-1} soil). The lowest *Azospirillum* population was observed in the non-rhizosphere soil of *Syzygium arnottianum* (5.0 x 10^4 CFU. g^{-1} soil).

3.7 *Beijerinckia*
Similar to bacteria and actinomycetes, the rhizosphere soil samples of *Pygeum gardneri* recorded the highest *Beijerinckia* population (32.5 x 10^5 CFU. g^{-1} soil). The minimum population of 0.5 x 10^5 CFU. g^{-1} soil was recorded in the non rhizosphere soil samples of *Syzygium arnottianum*. (Table 4).

3.8 Phosphate solubilizing microorganisms
The phosphate solubilizing microbial population *Pseudomonas, Fusarium* and *Aspergillus* in rhizosphere and non-rhizosphere soils of different shola species ranged between 1.0 x 10^6 CFU. g^{-1} soil and 7.0 x 10^6 CFU. g^{-1} soil. *Syzygium arnottianum* rhizosphere soils harboured the highest population of phosphate solubilizing microbes (11.1 x 10^6 CFU. g^{-1} soil).

3.9 AM Spore population and AM infection
The AM spore population ranged between 20 spores / 10 gram soil and 25 spores / 10 gram soil (Table 5). *Syzygium arnottianum* rhizosphere soils scored higher AM spores of 21 and AM infection of 45%, (Table 6) while *Pygeum gardneri* soil samples recorded the lowest AM spore population.

4. Discussion

4.1 Assessment of rhizosphere and non rhizosphere microflora of shola soils

The microbial diversity in rhizosphere soil collected from different shola plant species were compared with non-rhizosphere soil. In general, the density of bacteria and actinomycetes was higher in rhizosphere soils. Among the plant species *Pygeum gardneri* recorded maximum population of bacteria and actinomycetes the least in soils of *Syzygium arnottianum* plant species. In general, the actinomycetes population was found to be the least among the microbes analysed. This may be due to cool temperature and acidic environment prevalent in the hilly tracts. Similarly, the wide variation in bacterial and actinomycetes population between rhizosphere and non rhizosphere soils may be due to varied physico-chemical properties of the soils *viz*., pH, organic carbon and available nutrients. This is in accordance with the report of Vivekanandan *et al.* (1997) [19] that changes in average number of fungi and bacteria depend on soil-physico-chemical properties.

The qualitative analysis of microbial population revealed the presence of greater number of Gram Positive bacteria. In addition, many antagonistic bacteria were isolated from different locations.

The qualitative analysis of fungi revealed the occurrence of various fungal species like *Fusarium, Penicillium, Rhizopus* and *Trichoderma* (Table 2). Among these, *Fusarium* was the dominant genus in all locations followed by *Penicillium* and *Rhizopus.* Similarly, Baruah and Bora (1997) [3] studied species composition, population and biomass of microfungi in a tropical forest soil of Orissa and reported that *Aspergillus* and *Tricoderma viride* were the dominant genera among fungi imperfecti. Dominance of these fungi over other genera may be due to their high sporulating ability and rapid growth. Reddy (1962) [17] and Bor (1938) [5] recorded *Penicillium* as the dominant genus in the soils of Nilgiris high altitude forest. The results of the present study are agreement with the above observation. 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 (Ehrlich and Ehrlich, 1992) [7] was reported by several workers (Lavelle and Lepage, 1997) [12].

Even though plethora of reports (Dar *et al.* 1997, Janos, 1983) [6, 10] are available for microbial diversity of agricultural lands and grasslands, not much work has been done for shola forest tree species. The microbial diversity of few shola tree species of Longwood, Tiger hill and Thai shola of Nilgiris was reported by Homji (1965) [9].

Ehrlich and Ehrlich (1992) [7] reported microbial diversity of marine salterns of Gujarat using morphological, physiological and biochemical parameters. The leaf colonizing lichen diversity (folicolous) of Andaman Nicobar islands, Palani, Nilgiris hills and the north east was reported earlier (Homji (1965) [9], Janos (1983) [10]. Our results are in confirmity with these reports.

4.2 Microbes involved in nitrogen and phosphorus nutrition: The dinitrogen fixing bacterial isolates *viz*., *Azotobacter*, *Azospirillum* and *Beijerinckia* were obtained from shola plant species. Among the various diazotrophs, *Beijerinckia* dominated, since *Beijerinckia* is an acid preferring nitrogen fixer (Alexander, 1978) [1]

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*, *Beijerinckia* in various tropical forests has been reported by many workers (Balagoplan and Jose (1995) [2], Bonde and Rosswall (1987) [4] and Neal (1969) [15].

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 shola tree species whose available phosphorus content is low. 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. It was also observed that there were more number of fungal isolates than phosphate solubilizing bacteria. Based on morphological and cytological observations, the bacterial isolates were found to be *Pseudomonads* and fungal isolates were *Fusarium* and *Aspergillus*. Further, the phosphate solubilizing ability of these microbes was notice qualitatively by the formation of halo zone around colonies growing on Sperber's hydroxy apatite medium. The results of the study support the views of Jensen (1983) [11] that the phosphate solubilizing ability of the fungal isolates from tropical forests (Indonesia) are greater than bacterial strains.

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 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 *Syzygium arnottianum.*

4.3 AM infection

The AM fungal infection was greater in the root samples of *Syzygium arnottianum* over other plant species. Similarly, number of spores g^{-1} soil was too found to be the higher in the rhizosphere soil samples of *Syzygium arnottianum*. Highly fertile soils generally show less AM fungal population Marx (1991) [13], Mukeiji *et al.* (1996) [14]. So this, may account for lower number of AM spores and mycorrhizal infection in natural ecosystem over managed ecosystem Pacovsky (1986) [16] and Rhodes and Gerdemann (1975) [18]. The results of the present study may even be due to lack or insufficient number of viable spores.

Table 1: Dynamics of bacterial isolates in the Shola Rhizosphere soil

Shola plants	*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
Pygeum gardneri	23.0	-	33.0	1.5	1.0	-
Syzygium aronattianum	12.0	3.0	25.5	1.0	2.5	-

Table 2: Dynamics of fungal isolates in the Shola Rhizosphere soil

Shola plants	*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
Pygeum gardneri	7.00	3.00	1.00	-	4.00	1.00
Syzygium aronattianum	8.00	4.00	2.00	-	1.00	-

Table 3: Dynamics of Actinomycetes isolates in Shola Rhizosphere soil

Shola plants	(X x 10^6 CFU. g^{-1} soil)	
	R	S
Pygeum gardneri	7.00	16.50
Syzygium aronattianum	8.00	4.00

Table 4: Dynamics of Diazotrophic bacterial population of rhizosphere soils of different Shola species

Shola plants	*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
Pygeum gardneri	17.00	7.0	15.50	6.00	32.50	1.50
Syzygium aronattianum	9.50	2.50	4.50	5.00	13.50	0.5

Table 5: Occurrence of VAM spores in the rhizosphere soil of Shola species

Shola species	No of spores /10g soil
Pygeum gardneri	20
Syzygium aronattianum	21

Table 6: AM fungal infection in roots of Shola species

Shola species	Percent of infection*
Pygeum gardneri	31
Syzygium aronattianum	45

5. Conclusion

Research has revealed the presence of abundant microorganisms in Shola soil, and these studies have demonstrated their positive influence on the growth of regenerating seedlings. This signifies the potential of microbial inoculants to improve the development of tree seedlings, even in the absence of specific bio inoculants designed for Shola tree species. Therefore, it is crucial to conduct thorough assessments, documentations, and screenings to develop bio inoculants specifically tailored for Shola species. This study will greatly contribute to the successful establishment and management of Shola tree seeds and seedlings in nurseries. It can be inferred that microbial factors play a significant role in inducing germination and facilitating the establishment of Shola trees.

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