RESEARCH ARTICLE

BENEFICIAL MICROFLORA IN RHIZOSPHERE SOIL UNDER SELECTED EXOTIC FOREST TREE SPECIES

Tejkaran Patidar*, V. Jamaludheen, K. Surendra Gopal and Lakshmy A.

College of Forestry, KAU, Thrissur-680656 (Kerala), India
Email: teju.patel143@gmail.com

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Abstract: A field investigation was carried out with four exotic tree species (*Acacia auriculiformis, A. mangium, Casuarina equisetifolia* and *Swietenia macrophylla*) planted at 2m × 2m spacing and of about 30 years age at Kerala Forest Research Institute sub-centre Nilambur. The specific objective of the study was to examine the population variations of beneficial microflora in rhizosphere soil, due to long term occupancy of these trees. The rhizosphere soils were collected for isolation and enumeration of soil microflora like actinomycetes, bacteria, fungi, N-fixing bacteria, P-solubilizer and K-solubilising bacteria population. It was found that, over the years, the tree species influenced the soil microflora. The highest population of fungi, nitrogen fixing bacteria, phosphate solubilizing microorganism and potash solubilizing bacteria was recorded under *A. mangium*. The highest mean population of actinomycetes and bacteria was associated with *C. equisetifolia*. These four exotic tree species taken part actively in the improvement of soil quality and soil health which are the major determinants of sustainable soil productivity.

Keywords: Exotic forest tree species, Actinomycetes, Bacteria, Fungi, Beneficial microflora

INTRODUCTION

Soil productivity is defined as the capacity of soil, in its normal environment, to support plant growth. Soil productivity is reflected in the growth of forest vegetation or the volume of organic matter produced on a site. Soil is the fundamental resource of the forest. Without it or its productive capacity, the other resources of the forest are diminished. Soil characteristics such as physical, chemical and biological aspects are closely interrelated, and impacts on one aspects may influence others.

The type of organic compounds released by plants has been postulated to be a key factor influencing the diversity of microorganisms in the rhizosphere of different plant species (Bowen and Rovira, 1991). Rehabilitation activities do help increase the level of microbial community in the soils (Singh *et al.*, 2013). *Acacia mangium* has high nitrogen-fixing ability by symbioses with nodule forming bacteria and produces leaves that are more nitrogen-rich than native tropical leguminous trees (Tilki and Fisher, 1998). Lixia *et al.* (2004) observed that the total amount of microbes, especially of the number and percentage of bacteria was increased under artificial forests and most obvious result was found in soil under afforestation by *A. mangium*. They also suggest that the soil properties under artificial forests were improved because of microbes. *Casuarina equisetifolia* is a fast growing multipurpose actinorhizal tree capable of fixing atmospheric nitrogen by virtue of a symbiotic relationship between the plant roots and *Frankia* (Rajendran and Devaraj, 2004). *Swietenia macrophylla* is a promising structured timber species that was introduced to Indian sub-continents long back. The biomass and nutrient accumulation in exotics like *Swietenia macrophylla* has greater acclimation capacity to enhance the photosynthetic plasticity under full sun.

From the voluminous literature on rhizosphere, it is known that the microbial community is not only influenced by root exudates and nutrient uptake but also by the plant species (Smalla *et al.*, 2001; Wieland *et al.*, 2001), phenology (Dunfield and Germida, 2003; Elsas *et al.*, 2008), the background soil characteristics (Marschner *et al.*, 2001; Buckley and Schmidt, 2003; Girvan *et al.*, 2003; Johnson *et al.*, 2003), and the developmental stage of the plants (DiCello *et al.*, 1997).

In this context, understanding the diversity and dynamics of microflora population will throw light into various aspects of possible soil productivity changes over time due to long occupancy of exotic tree species. Moreover, the possible effect of different tree species on the soil microflora will give valuable information to the overall micro site enrichment potential of these exotic trees. However, the information on the potential for soil productivity improvement in relation to the microbial diversity are scarce from these exotic species. Hence, the present study was undertaken with the specific objective to study the variations in soil productivity due to long term occupancy of selected exotic tree species with special reference to the quantity and quality of the soil microflora.

MATERIAL AND METHOD

Experimental site

The proposed study was conducted in an existing provenance trial plot of selected exotic tree species located in the sub-centre of Kerala Forest Research Institute, at Nilambur. The site is located in

*Corresponding Author

Malappuram district of Kerala State at 11°16′37″N latitude and 76°13′33″E longitude. The area has an elevation of 41 m above mean sea level. The temperature varies between 17^{o} C and 37^{o} C and the mean annual rainfall ranges from 2500 to 3500 mm.

Species descriptions

The following four fast growing exotic tree species were selected for the study

Acacia auriculiformis, Casuarina equisetifolia and *Swietenia macrophylla* were planted during 1982 and *Acacia mangium* was planted during 1984 with a spacing of 2m × 2m.

Collection of Soil samples

The soil samples for microbial analysis were collected from the rhizosphere, within a radius of 30 cm distance from the tree and from depth of 0-30cm layer of the top soil. Three replicate soil samples were collected from the plots of all the four tree species. At each sampling, 15 samples were collected (4 tree species + tree less open area x 3 replications) for soil microflora analysis.

Isolation and enumeration of total soil microflora

Total bacteria, fungi, actinomycetes, nitrogen fixing bacteria (NFB), phosphate solubilizing microorganism (PSM), and potash solubilizing bacteria (KSB) were isolated and enumerated. Quantitative estimation of bacteria, fungi, actinomycetes, NFB, PSM and KSB from different soil samples were carried out on Nutrient Agar medium, Rose Bengal Agar medium, Kenknights medium (Johnson and Curl, 1972), Jensen's Broth, Pikovskaya Agar medium (Rao and Sinha, 1963), and Glucose Yeast Extract Agar medium respectively by using serial dilution method. Serial dilution-agar plating method (or viable plate count method) was used for isolation and enumeration of microorganisms from soil. Different dilutions were used for isolation of different microflora. The dilutions of 10^{-2} is selected for fungi and nitrogen fixing bacteria, 10^{-3} for actinomycetes, phosphate solubilizing microorganism and potash solubilizing bacteria and 10^{-4} for bacteria.

Statistical analysis

Statistical analysis was done by using statistical software SPSS V.20.0. Duncan’s Multiple Range Test (DMRT) was used to test the differences among treatment means.

RESULT

In the present investigation isolation and enumeration of beneficial microflora viz., actinomycetes, bacteria, fungi, nitrogen fixing bacteria, phosphate solubilizing microorganism and potash solubilizing bacteria were carried out. The results of the summer season and rainy season are detailed hereunder. The population of microflora was expressed as a colony forming units (CFUs).

Summer (May 2013)

The tree species had influenced the microbial population. Among all the tree species *Casuarina equisetifolia* had highest actinomycetes population (6.33×10^{3}cfu g^{-1}) followed by *Acacia auriculiformis, Acacia mangium* and *Swietenia macrophylla* (Table 1). The lowest actinomycetes population was recorded in treeless control plot. It was found that all tree species did not differ significantly in the bacterial and fungal population. The bacterial population was highest in *A. mangium* plantation (18.33×10^{4} cfu g^{-1}) and the lowest in *S. macrophylla* (10.00×10^{4} cfu g^{-1}). However, *C. equisetifolia, A. auriculiformis,* treeless control plot and *S. macrophylla* were at par with *A. mangium*. The fungal population was highest in *Acacia mangium* plantation (33.00×10^{2} cfu g^{-1}) and lowest in treeless control plot (18.33×10^{2} cfu g^{-1}). *A. mangium, A. auriculiformis, C. equisetifolia, S. macrophylla* and treeless control plot had a moderate fungal population. The nitrogen fixing bacteria (NFB) was highest in *Acacia mangium* plantation (4.67×10^{2} cfu g^{-1}) followed by *A. auriculiformis,* treeless control plot and *C. equisetifolia.* It was lowest in *S. macrophylla* (1.33×10^{2} cfu g^{-1}). The population of phosphate solubilizing microorganism (PSM) in all the plantations were significantly higher than treeless control plot. The population was highest in *Acacia mangium* plantation (15.33×10^{3} cfu g^{-1}) and lowest in tree less treeless control plot (4.33×10^{3} cfu g^{-1}). *Acacia mangium* and *A. auriculiformis* had high potash solubilizing bacteria (KSB) and it was significantly higher from other plantations and treeless control plot. The population of KSB was highest under *Acacia mangium* (23.67×10^{3} cfu g^{-1}) and lowest in treeless control plot (3.33×10^{3} cfu g^{-1}).

Table 1. Microbial population under different exotic tree species during summer season (May, 2013).

Treatment	Actinomycetes (x 10^{3} cfu g^{-1})	Bacteria (x 10^{4} cfu g^{-1})	Fungi (x 10^{2} cfu g^{-1})	NFB (x 10^{2} cfu g^{-1})	PSM (x 10^{3} cfu g^{-1})	KSB (x 10^{3} cfu g^{-1})
Acacia auriculiformis	5.33^{ab} (1.15)	13.00 (2.00)	28.33 (10.40)	4.00^{a} (1.00)	7.66^{ab} (3.05)	19.67^{a} (6.02)
Acacia mangium	5.33^{ab} (1.52)	18.33 (4.04)	33.00 (13.00)	4.67^{a} (1.15)	15.33^{a} (1.52)	23.67^{a} (6.42)
Casuarina equisetifolia	6.33^{a} (1.52)	16.67 (6.65)	24.33 (3.21)	2.33^{ab} (0.57)	12.00^{bc} (3.60)	03.67^{b} (2.88)
Swietenia macrophylla	5.00^{ab} (0)	10.00 (1.00)	20.33 (1.52)	1.33^{b} (0.57)	09.00^{b} (1.00)	06.67^{b} (1.52)
Treeless control plot	3.33^{b} (0.57)	12.67 (6.11)	18.33 (01.15)	3.33^{ab} (2.08)	04.33^{c} (2.08)	03.33^{b} (1.52)

Values in the parenthesis are standard deviation of the mean.
Values followed by same superscript in a column do not differ significantly (LSD, P, 0.05).

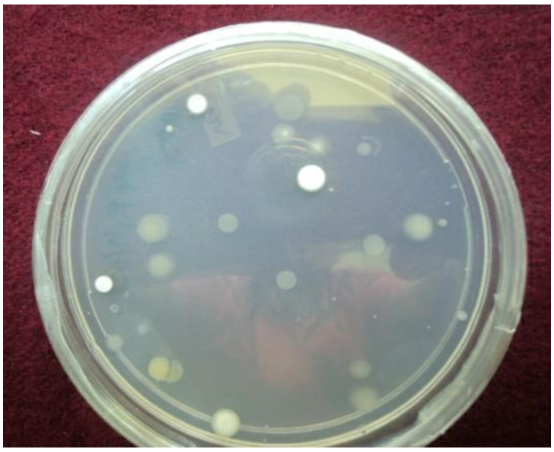

Plate 1: Actinomycetes

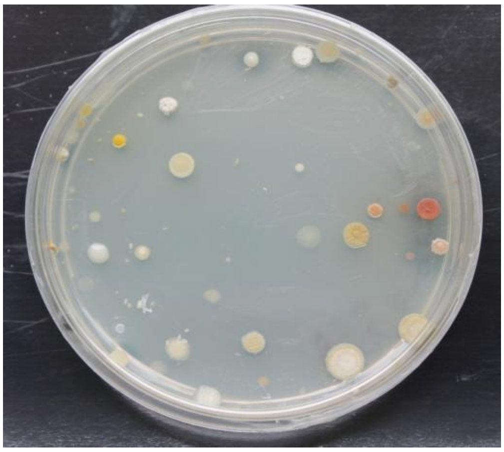

Plate 2: Bacteria

Plate 3: Fungi

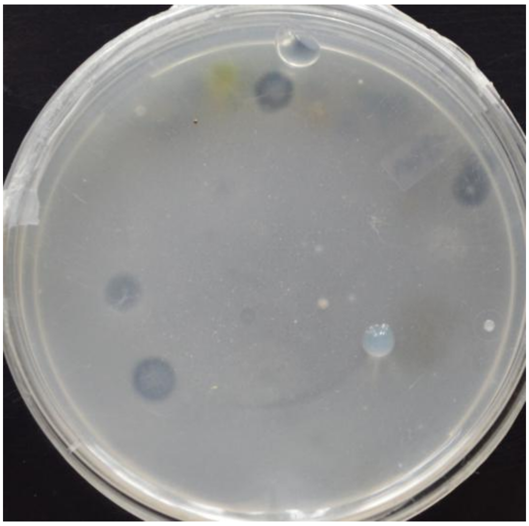

Plate 4: Nitrogen fixing bacteria

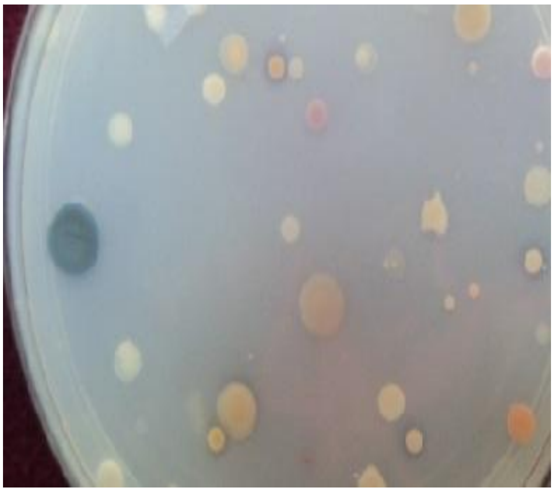

Plate 5: Phosphate solubilizing microorganism

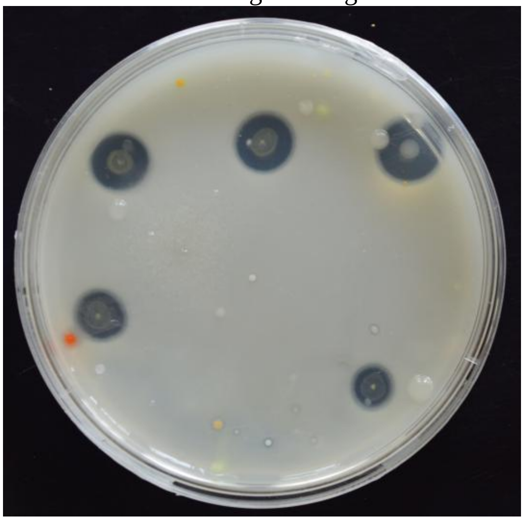

Plate 6: Potassium solubilizing bacteria

Rainy season (August 2013)

The soil sample for second quarter collected in August 2013 showed that tree species had influence on microbial population (Table 2). The population of actinomycetes was highest in *Casuarina equisetifolia* (4.33×10^3 cfu g^{-1}) and lowest in *S. macrophylla* (1.67×10^3 cfu g^{-1}). The bacterial population was significantly higher in plantations compared to treeless control plot. The bacterial population was highest in *C. equisetifolia* (35.33×10^4 cfu g^{-1})

followed by *A. auriculiformis* (21.22 × 10^4 cfu g^{-1}), *A. mangium* (19.33 × 10^4 cfu g^{-1}) and *S. macrophylla* (11.00 × 10^4 cfu g^{-1}). The bacterial population was lowest in treeless control plot (2.33 × 10^4 cfu g^{-1}). Fungal population in *A. auriculiformis* plantation (19.00 × 10^2 was significantly higher than other treatments and it was least in treeless control plot (1.67 × 10^2 cfu g^{-1}). The population of nitrogen fixing bacteria was highest in *A. auriculiformis* (1.67 × 10^2 cfu g^{-1}) followed by *A. mangium* (1.33 × 10^2 cfu g^{-1}) and *C. equisetifolia* (1.33 × 10^2 cfu g^{-1}). The nitrogen fixing bacteria population was lowest in treeless control plot (0.67 × 10^2 cfu g^{-1}). Phosphate solubilizing microorganism population was significantly higher in *A. mangium* plantation (7.33 × 10^3 cfu g^{-1}) than treeless control plot (2.00 × 10^3 cfu g^{-1}). The phosphate solubilizing microorganism population in *A. mangium* was at par with *S. macrophylla, C. equisetifolia* and *A. auriculiformis.* The population of KSB was significantly higher in plantations than treeless control plot. The KSB population recorded highest in *A. mangium* (4.33 × 10^3 cfu g^{-1}) and it was recorded lowest in treeless control plot (1.00 × 10^3 cfu g^{-1}).

Table 2. Microbial population under different exotic tree species during rainy season (August, 2013).

Treatment	Actinomycetes (x 10^3 cfu g^{-1})	Bacteria (x 10^4 cfu g^{-1})	Fungi (x 10^2 cfu g^{-1})	NFB (x 10^2 cfu g^{-1})	PSM (x 10^3 cfu g^{-1})	KSB (x 10^3 cfu g^{-1})
Acacia auriculiformis	2.67 (1.52)	21.33^{b} (3.21)	19.00^{a} (7.81)	1.67 (0.57)	2.67^{ab} (0.57)	3.67^{a} (0.57)
Acacia mangium	3.00 (2.64)	19.33^{bc} (7.57)	9.00^{b} (2.64)	1.33 (0.57)	7.33^{a} (5.03)	4.33^{a} (0.57)
Casuarina equisetifolia	4.33 (1.15)	35.33^{a} (8.32)	8.33^{b} (2.51)	1.33 (0.57)	4.00^{ab} (1.00)	4.00^{a} (1.00)
Swietenia macrophylla	1.67 (1.15)	11.00^{cd} (2.00)	2.67^{b} (1.52)	1.00 (1.00)	6.33^{ab} (1.15)	3.00^{a} (1.00)
Treeless control plot	2.33 (0.57)	2.33^{d} (1.52)	1.67^{b} (0.57)	0.67 (0.57)	2.00^{b} (1.00)	1.00^{b} (1.00)

Values in the parenthesis are standard deviation of the mean.
Values followed by same superscript in a column do not differ significantly (LSD, P, 0.05).

DISCUSSION

Exotic forest tree species in the recent years has achieved wide acceptance in the plantation programmes in Kerala, owing to their multi-purpose and fast growing nature. As invasive species these species have might some effect on soil habitat and it is the basic requisite and very much important to examine the soil productivity changes under exotic forest tree species. The present study is undertaken to study the population of beneficial microflora in rhizosphere soil under exotic forest tree species. The tree plots showed rich microfloral population compared to the contiguous treeless control plot. Thus the selection of suitable exotic species is very important not only to obtain the high yield but also to maintain soil productivity. The changes in beneficial microflora population of soil under the selected exotic forest tree species viz. *A. auriculiformis, A. mangium, C. equisetifolia* and *S. macrophylla* is discussed hereunder in detail.

In general, the present study showed that the microbial population was higher in tree plots compared to treeless control plot. In summer season, *A. mangium* was found dominant species among the tree species and among the microflora fungi population was highest. Highest population of fungi was also supported by Rodrigues *et al*. (2011). Rodrigues *et al*. (2011) reported that fungi developed better during the dry season and bacteria during rainy season. *C. equisetifolia* had highest population of actinomycetes it might be because of symbiotic relationship of *C. equisetifolia* with actinomycetes. *A. mangium* had highest population of bacteria, fungi, nitrogen fixing bacteria, phosphorus and potash solubilizing bacteria which might be because of high nitrogen fixing capacity of legume tree species and addition of litterfall. A fairly high N-fixing potential also has been recorded for *Acacia mangium* by Gueye and Ndoye, (1998). The highest microbial population in *A. mangium* is supported by Bakarr and Janos, (1996). They also found the higher number of microorganism colonization in *A. mangium.*

In the rainy season, among all the microflora bacteria population was highest. *C. equisetifolia* was found dominant species and it had highest population of actinomycetes and bacteria. It might be because of symbiotic relation with microorganism which is found in *C. equisetifolia*. *A. auriculiformis* had highest fungi and nitrogen fixing bacterial population. *A. mangium* had highest phosphorus solubilizing microorganism and KSB population which was found in previous sampling also. As shown earlier, this higher number of microbial population in tree species might be because of litterfall, symbiotic relation of microorganism with tree species and microclimate developed in rhizosphere (root soil) soil by tree species (Sharma *et al.*, 2009; Golinska and Dahm, 2011). Silva *et al.*, (2005) found that microbial population were significantly ($p<0.01$) greater in forest soil than in old-field soil, which could also be related to the higher level of soil organic matter in the forest soil.

Consequent to the high microbial population particularly the beneficial microflora, the tree plots in general accrued good growth and in turn, maintained the soil condition due to long term occupancy of these exotic tree species. The potential ability of the exotic fast growing multipurpose tree species for improving the soil productivity and soil health is highlighted throughout the course of this investigation.

CONCLUSION

The higher microbial population under the trees compared to the treeless control plots clearly implies the potential ability of these exotic tree species for improving the soil qualities when the beneficial microflora are taken as indices of soil productivity. Among all the tree species *A. mangium* had highest mean population of fungi, nitrogen fixing bacteria, phosphate solubilizing microorganism and potash solubilizing bacteria. The highest mean population of actinomycetes and bacteria were associated with *C. equisetifolia*. These exotic tree species taken part actively in the improvement of soil quality and soil health which are the major determinants of sustainable soil productivity. In Future detailed studies on microflora at species level may be taken up. Beneficial effect of microflora may be examined. Litter decomposition and dynamics should may be in cooperated along with the microflora studies.

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