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Dynamics of microbial communities in red sanders (*Pterocarpus santalinus*) rhizosphere soil

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ABSTRACT

The rhizosphere soil microbial dynamics and fertility status of the red sanders at different locations viz Rangapuram, Athiyandal, Karumandurai, Balapuram and Attur were studied. In order to find out the impact of Red Sanders on soil microbes, the microbial dynamics and fertility status of non-rhizosphere soils were also assessed. In general, the dynamics of microflora varied widely among locations as well as between red sanders rhizosphere and non-rhizosphere soils. The results showed that Balapuram rhizosphere was most rich in bacterial but poor in fungal population; Rangapuram rhizosphere soils were most rich in actinomycetes but poor in bacterial population and Attur rhizosphere soils were most rich in fungal but poor in actinomycetes population, among the five locations. The mean rhizosphere soils and non-rhizosphere soils ratio of microflora was maximum (1.64) at Rangapuram. Highest pH of 6.57 each was recorded in rhizosphere soils at Karumandurai and Attur, EC in rhizosphere soils (1.10 dS/m) at Athiyandal and organic carbon in rhizosphere soils at Athiyandal and Balapuram (0.87% each). Maximum available N in rhizosphere soils (459.94 kg/ha) was at Karumandurai; available P in rhizosphere soils (16.42 kg/ha) at Attur and available K in rhizosphere soils (280.00 kg/ha) at Karumandurai. While comparing rhizosphere and non-rhizosphere soils, it was found that non-rhizosphere soils were microbially more diverse. The beneficial microbes viz *Azotobacter, Azospirillum* and phosphate solubilizing microorganisms were isolated from soil samples collected from different locations.

Keywords: Red sanders; microbial communities; rhizosphere; non-rhizosphere

INTRODUCTION

Microorganisms constitute less than 0.5 per cent (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) (Wall and Virginia 1999) and are responsible for the cycling of organic compounds. Soil microorganisms also influence above ground ecosystems by contributing to plant growth and soil fertility (Yao et al 2000). 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 micro-biota, through mismanagement or environmental interferences results in decline or even death of the above ground plant population.

An understanding of soil microbes, their properties and the nature of the interaction with and within their environment is essential. With this background, the study on assessment of rhizosphere microbial diversity of red sanders was undertaken.

Pterocarpus santalinus popularly known as red sanders (Fabaceae) is a tropical dry deciduous forest species. Red sanders is an endangered timber tree species, endemic to southern India. It grows in approximately 5,160 km^2 of fragmented forest landscape of southern Andhra Pradesh and in a few sporadic patches in Tamil Nadu and Karnataka states.

The wood is primarily used for making musical instruments and luxury furniture. It also yields santalin dye (Ferreira et al 2004) which finds use in colouring foodstuff (Mulliken and Crofton 2008) and pharmaceutical preparations (Schmidlin et al 2008). Additionally, the extracts obtained from the tree bark and wood are known to have several medicinal properties (Saikia et al 2006). The tree can grow only in dry plains of India like eastern Ghats and eastern plains of Karnataka like Kolar, Tumkur and Chitradurga. It grows in parts of Tirupati hills and Srisailam in Andhra Pradesh (Vedavathy et al 1997). It prefers lateritic and gravelly soil and cannot tolerate water logging. In natural habitat, the tree experiences hot, dry climate with normal rainfall of 88-105 cm received from northeastern and southwestern monsoons.

Red sanders has the prominent importance in the business. Research initiatives are under way to exploit its potential application in soaps, dyes, toys etc. Despite a ban on exploitation and sale, good quality red sanders fetched any price between Rs 3 and 4 lakhs per tonne in the global market, while the inferior type was sold as fuelwood like other timbers (Bhagyaraj and Ramana 2013). Red sanders has fallen back into the 'endangered' category in the International Union for Conservation of Nature's (IUCN) Red List (Jha 2022).

The red sanders wood has different uses in traditional and folklore medicines and is used for the treatment of diabetes, prickly heat, skin diseases, ulcers, eye diseases inducing vomiting, mental aberrations, snake bites, scorpion stings and for various other ailments. The heartwood is known to have anti-hyper glycemic activity; is anti-pyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac and diaphoretic activities and is a cooling agent (Ramabrahmam and Sujatha 2016).

MATERIAL and METHODS

Red sanders rhizosphere soil (R) samples were collected from different locations viz Rangapuram, Athiyandal, Karumandurai, Balapuram and Attur. The samples were packed in sterile polythene bags and brought to the laboratory for further analysis.

For comparison, non-rhizosphere soil samples (S) adjacent to red sanders plantations were also collected and analysed for microbial population. The samples were collected from each location during March 2023, packed in sterile polythene bags and brought to the laboratory for further analysis. These were stored at 4°C till processed for further biological and physico-chemical properties.

Serial dilution of the samples: 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 were enumerated by plating one ml of 10^{-5} dilution in the sterile Petri dishes using nutrient agar medium. The colonies appearing on the plate after 48 h of incubation at 30°C were counted and expressed as number of CFU per g of soil. Fungi were enumerated by plating one ml of 10^{-4} 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 number of CFU per g of soil. For actinomycetes, one ml of 10^{-6} 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 number of CFU per g of soil.

Isolation of diazotrophic and phosphate solubilizing microorganisms: The dinitrogen utilizing microbes such as *Azotobacter, Azospirillium* and phosphate solubilizing microorganisms were isolated from rhizosphere soil samples collected from various locations viz Rangapuram, Athiyandhal, Karumandurai, Balapuram and Attur.

RESULTS and DISCUSSION

Data in Table 1 show that among the locations, Balapuram rhizosphere soils recorded maximum population of bacteria (31.0×10^5 CFU/g soil) and Rangapuram the minimum (16.0×10^5 CFU/g soil). The difference between bacterial population in rhizpsphere and non-rhizosphere soils was also the highest at Balapuram (11.0×10^5 CFU/g soil) and lowest at Rangapuram and Karumandurai (6.0×10^5 CFU/g soil each). The highest fungal population was recorded in the rhizosphere soils at Attur ($33.0 \text{ x } 10^4$ CFU/g soil) and the lowest at Balapuram ($21.0\ 10^4$ CFU/g soil). The difference between fungal population in rhizosphere and non-rhizosphere soils was also

Table 1. Microflora of red sanders rhizosphere and non-rhizosphere soils of different locations

Location	Microflora								
	Bacteria ($\times 10^5$ CFU/g soil)			Fungi ($\times 10^4$ CFU/g soil)			Actinomycetes ($\times 10^6$ CFU/g soil)		
	R	S	R – S	R	S	R – S	R	S	R – S
Rangapuram	16.0	10.0	6.0	31.0	17.0	14.0	24.0	16.0	8.0
Athiyandal	29.0	21.0	8.0	28.0	18.0	10.0	13.0	9.0	4.0
Karumandurai	22.0	16.0	6.0	22.0	13.0	9.0	22.0	15.0	7.0
Balapuram	31.0	20.0	11.0	21.0	14.0	7.0	18.0	13.0	5.0
Attur	24.0	17.0	7.0	33.0	20.0	13.0	10.0	7.0	3.0
Mean	24.4	16.8		27.0	16.4		17.4	12.0	

R: Rhizosphere soils, S: Non-rhizosphere soils

highest at Rangapuram (14.0 × 10^4 CFU/g soil) and the lowest at Balapuram (7.0 × 10^4 CFU/g soil). Maximum population of actinomycetes was observed in rhizosphere soils at Rangapuram (24.0 x 10^6 CFU/g soil) and minimum at Attur (10.0 x 10^6 CFU/g soil). The difference between actinomycetes population in rhizosphere and non-rhizosphere soils was also recorded highest at Rangapuram (8.0 x 10^6 CFU/g soil) and lowest at Attur (3.0 x 10^6 CFU/g soil).

The results show that Balapuram rhizosphere and non-rhizosphere soils were most rich in bacterial but poor in fungal population; Rangapuram rhizosphere and non-rhizosphere soils were most rich in actinomycetes but poor in bacterial population and Attur rhizosphere and non-rhizosphere soils were most rich in fungal but poor in actinomycetes population, among the five locations.

Data in Table 2 depict that the bacterial population of *Azototobacter*, *Azospirillum* and *Phosphobacteria* was recoded maximum 31.0, 36.0 and 34.0 × 10^5 CFU/g soil in the rhizosphere soils at Athiyandal, Balapuram and Attur and minimum 14.0, 18.0 and 17.0 at Rangapuram, Attur and Athiyandal respectively. Thus the *Azototobacter* population was highest and *Phosphobacteria* population was lowest in the rhizosphere soils of Athiyandal, whereas, *Phosphobacteria* population was highest and *Azospirillum* population was lowest in the rhizsphere soils of Attur, among all the locations.

Data given in Table 3 exhibit that the mean rhizosphere and non-rhizosphere soils ratio of microflora was maximum (1.64) at Rangapuram and minimum (1.44) at Athiyandal. The rhizosphere and non-rhizosphere soils ratio of fungi and bacteria was maximum at Rangapuram (1.82 and 1.60 respectively). The rhizosphere and non-hizosphere soils ratio of fungi was minimum (1.50) at Balapuram and of bacteria at Karumandurai (1.37). The rhizosphere and non-rhizosphere soils ratio of actinomycetes was maximum (1.80) at Karumandurai and minimum at Athiyandal, Balapuram and Attur (1.40 each).

Highest pH of 6.57 each was recorded in rhizosphere at Karumandurai and Attur and lowest 6.41 at Balapuram (Table 4). The difference between pH of rhizosphere and non-rhizosphere soils was highest (0.38) at Rangapuram and lowest (0.08) at Balapuram. Highest EC in rhizosphere soils and difference between EC of rhizosphere and non-rhizosphere soils (1.10 and 0.36 dS/m respectively) were recorded at Athiyandal and lowest (0.79 and 0.06 dS/m respectively) at Karumandurai. Organic carbon in rhizosphere soils was recorded maximum at Athiyandal and Balapuram (0.87% each) and difference between organic carbon of rhizosphere and non-rhizosphere soils was maximum (0.17%) in rhizosphere soils of Athiyandal. The difference between organic carbon of rhizosphere and non-rhizosphere soils was minimum at Karumandurai and Balapuram (0.10% each).

Data in Table 5 indicate that available N in rhizosphere soils was maximum (459.94 kg/ha) at Karumandurai and minimum at Rangapuram (192.33 kg/ha); the difference in available N between rhizosphere and non-rhizosphere soils was maximum at Attur (115.05 kg/ha) and minimum at Athiyandal (27.70 kg/ha). Available P in rhizosphere soils was

Table 2. Diazotrophic bacterial population and phosphate solubilizing microbial population of red sanders rhizosphere soils of different locations

Location	Bacterial population (× 10^5 CFU/g soil)		
	Azototobacter	*Azospirillum*	*Phosphobacteria*
Rangapuram	14.0	31.0	23.0
Athiyandal	31.0	26.0	17.0
Karumandurai	17.0	26.0	25.0
Balapuram	25.0	36.0	23.0
Attur	25.0	18.0	34.0
Mean	22.4	27.4	24.4

Table 3. Ratio of microbes of rhizosphere (R) and non-rhizosphere soils (S) at different locations

Location	R-S ratio of microflora			
	Fungi	Bacteria	Actinomycetes	Mean
Rangapuram	1.82	1.60	1.50	1.64
Athiyandal	1.55	1.38	1.40	1.44
Karumandurai	1.69	1.37	1.80	1.62
Balapuram	1.50	1.55	1.40	1.48
Attur	1.65	1.41	1.40	1.48
Mean	1.64	1.46	1.50	1.53

Table 4. Physico-chemical properties of rhizosphere of red sanders and adjacent non-rhizosphere soil

Location	Property								
	pH			EC (dS/m)			Organic carbon (%)		
	R	S	R – S	R	S	R – S	R	S	R – S
Rangapuram	6.51	6.13	0.38	1.03	0.70	0.33	0.72	0.61	0.11
Athiyandal	6.49	6.35	0.14	1.10	0.74	0.36	0.87	0.70	0.17
Karumandurai	6.57	6.44	0.13	0.79	0.73	0.06	0.55	0.45	0.10
Balapuram	6.41	6.33	0.08	0.91	0.81	0.10	0.87	0.77	0.10
Attur	6.57	6.41	0.16	1.03	0.91	0.12	0.60	0.47	0.13
Mean	6.51	6.332		0.972	0.778		0.722	0.6	

R: Rhizosphere soils, S: Non-rhizosphere soils

maximum (16.42 kg/ha) at Attur and minimum at Rangapuram and Balapuram (11.94 kg/ha each); the difference in available P between rhizosphere and non-rhizosphere soils was maximum at Attur (3.00 kg/ha) and minimum at Balapuram (0.37 kg/ha). Available K in rhizosphere soils was maximum (280.00 kg/ha) at Karumandurai and minimum at Attur (238.93 kg/ha); the difference in available K between rhizosphere and non-rhizosphere soils was maximum at Balapuram (33.74 kg/ha) and minimum at Attur (21.47 kg/ha).

The higher number and occurrence of more microflora in the rhizosphere soil compared to non-rhizosphere soil was reported by Olahan et al (2016) in jute and Bopaiah and Shetty (1991) in pepper, cacao, pineapple and coconut. Li et al (2016) reported that all

Table 5. Nutrient status of red sanders rhizosphere and nearby non-rhizosphere soil

Location	Nutrient (kg/ha)								
	Available N			Available P			Available K		
	R	S	R – S	R	S	R – S	R	S	R –S
Rangapuram	192.33	158.53	33.80	11.94	11.01	0.93	261.33	230.60	30.73
Athiyandal	309.41	281.71	27.70	12.69	10.97	1.72	253.86	221.63	32.23
Karumandurai	459.94	349.31	110.63	14.93	12.23	2.70	280.00	250.40	29.60
Balapuram	309.41	220.80	88.61	11.94	11.57	0.37	257.60	223.86	33.74
Attur	451.58	336.53	115.05	16.42	13.42	3.00	238.93	217.46	21.47
Mean	344.53	269.38	75.15	13.58	11.84	1.74	258.34	228.79	29.55

R: Rhizosphere soils, S: Non-rhizosphere soils

soil nutrient contents and microbial diversity indices were higher in rhizosphere soil compared to non-rhizosphere soil. Aparna et al (2020) also found that the microflora was more in rhizosphere than in the non-rhizosphere.

The photosynthetically fixed carbon that enters the soil ecosystem as plant biomass and root exudates is the prime source of energy for microbes. Hence, maximum microbial activity occurs in an ecosystem encompassed by the soil and root interface that is rhizosphere. Thus the provision of energy to the microbial community by root exudates, dead roots and sloughed cells results in an intense microbial activity and microbial interaction in rhizosphere over non-rhizosphere soils. The quality and quantity of microbial community changes with the growing season of the plants, kind of plants and locations. Hence, microbial habitats are produced continually through root growth. Thus understanding the biological activities of a particular rhizosphere provides a model for successful modification of native microbial community dynamics.

The microbial dynamics in red sanders rhizosphere soils were collected from various locations and compared with non-rhizosphere soils. In general, the density of bacteria, fungi and actinomycetes was higher in rhizosphere soils.

The soils of Attur had the lowest population of actinomycetes owing to their acidic pH (Meiklejohn 1957, Alexander 1977).

The wide variation between bacterial and actinomycetes population among locations and between rhizospher and non-rhizospher soils might be due to varied physico-chemical properties of the soils viz pH, organic carbon and available nutrients. This finding is in accordance with the report of Shail and Dubey (1997) who reported that changes in average number of fungi and bacteria in Kumaun Himalaya soils were positively correlated with soil moisture and negatively with soil pH.

The highest available nitrogen and organic matter content of the Athiyandal and Balapuram soils might be responsible for greater bacterial and actinomycetes observed in the study area. The dinitrogen fixing bacterial isolates viz *Azotobacter* and *Azospirillum* were obtained from various locations of red sanders rhizosphere. Among the various diazotrophs, *Azospirillum* dominated at all the locations except Attur since *Azospirillum* is a neutral preferring nitrogen fixer (Alexander 1977).

Athiyandal soils harboured greater number of *Azotobacter* which could be due to higher organic matter content of the Athiyandal soils. Similar to the present study, the occurrence of *Azotobacter* and *Azospirillum* in various tropical forests has been reported by many workers (Nagaraj 1989, Paul 1999, Venkatachalam 2003, Vennila et al 2023).

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 investigations, maximum phosphate solubilizing microbes were obtained from the soils of Attur whose available phosphorus content was the least among the locations studied.

The rhizosphere effect or the degree of stimulation of an activity of population by the plant root was described by evaluating the ratio of activity per unit weight of rhizosphere soil and the activity per unit weight of non-rhizosphere soil. The values greater than one indicate selective stimulation in the rhizosphere; equivalence of the two activities suggests no rhizosphere effect, whereas, R/S ratio of less than one reveals inhibition of the activity in the rhizosphere. In the current study, the R/S ratio of microbes' population was more than one at all locations. This might be due to the presence of numerous antagonistic bacteria at this location. Even though the values are more than one, the ratio should be more than 10 for their effective activity (Rao 1997).

CONCLUSION

It can be concluded that there were differences in bacterial, fungal and actinomycetes population size in rhizosphere and non-rhizosphere soils of red sanders. In general, the dynamics of microflora varied widely among locations as well as between red sanders rhizosphere and non-rhizosphere soils. The density of bacteria was found to be higher in Balapuram rhizosphere and fungal population in Attur rhizosphere soils. Among the red sanders rhizosphere, Athiyandal harboured greater number of microbial population while Rangapuram and Balapuram rhizosphere soils exhibited on bar microbial dynamics. Comparing rhizosphere and non-rhizosphere soils, non-rhizosphere soils were microbially more diverse. The knowledge obtained from the present study would be of help in understanding the natural changes of the microbial population size in rhizosphere soils under red sanders.

REFERENCES

Alexander M 1977. Introduction to soil microbiology, 2nd Edn. John Wiley Eastern Limited, New Delhi, India, 467p.

Aparna B, Gladis R, Joseph B and Sathyan NR 2020. Comparative study on the dynamics of rhizosphere and non-rhizosphere soil. International Journal of Current Microbiology and Applied Sciences **9(6):** 2527-2535.

Bhagyaraj A and Ramana DV 2013. Status of red sanders in business. Indian Journal of Applied Research **3(7):** 405-407.

Bopaiah BM and Shetty HS 1991. Soil microflora and biological activities in the rhizospheres and root regions of coconut-based multi-storeyed cropping and coconut monocropping systems. Soil Biology and Biochemistry **23(1):** 89-94.

Ferreira ESB, Hulme AN, McNab H and Quye A 2004. The natural constituents of historical textile dyes. Chemical Society Reviews **33(6):** 329-336.

Jha S 2022. Red sanders falls back in IUCN's 'endangered' category. DownToEarth, 10 January 2022.

Li Z, Zu C, Wang C, Yang J, Yu H and Wu H 2016. Different responses of rhizosphere and non-rhizosphere soil microbial communities to consecutive *Piper nigrum* L monoculture. Science Reporter **6:** 35825: 10.1038/srep35825.

Meiklejohn J 1957. Numbers of bacteria and actinomycetes in a Kenyan soil. European Journal of Soil Science **8(2):** 240-247.

Mulliken T and Crofton P 2008. Review of the status, harvest, trade and management of seven Asian CITES-listed medicinal and aromatic plant species: results of the R+D project FKZ 804 86 003. Bundesamt für Naturschutz (BfN), Federal Agency for Nature Conservation Konstantinstrasse, Bonn, Germany, 142p.

Nagaraj R 1989. Occurrence of *Azotobacter* and *Beijerinckia* in forest soils of Maharashtra. Indian Journal of Forestry **12(2):** 112-116.

Olahan GS, Sule IO, Garuba T and Salawu YA 2016. Rhizosphere and non-rhizosphere soil mycoflora of *Corchorus olitorius* (jute). Science World Journal **11(3):** 23-26.

Paul P 1999. Soil biota in sandal seedling establishment. MSc Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Ramabrahmam V and Sujatha 2016. Red sanders in Rayalaseema region of Andhra Pradesh: importance to commercial and medicinal value. IOSR Journal of Pharmacy and Biological Sciences **11(1):** 57-60.

Rao NSS 1997. Soil microorganisms and plant growth. Oxford and IBH Publishing Company Private Limited, New Delhi, India.

Saikia AP, Ryakala VK, Sharma P, Goswami P and Bora U 2006. Ethnobotany of medicinal plants used by Assamese people for various skin ailments and cosmetics. Journal of Ethnopharmacology **106(2):** 149-157.

Schmidlin L, Poutaraud A, Claudel P, Mestre P, Prado E, Santos-Rosa M, Wiedemann-Merdinoglu S, Karst F, Merdinoglu D and Hugueney P 2008. A stress-inducible resveratrol O-methyltransferase involved in the biosynthesis of pterostilbene in grapevine. Plant Physiology **148(3):** 1630-1639.

Shail S and Dubey RC 1997. Seasonal changes in microbial community in relation to edaphic factors in two forest soils of Kumaun Himalaya. In: Recent researches in ecology, environment and pollution, Vol XI (SC Sati, J Saxena and RC Dubey, Eds), Today and Tomorrow's Printers and Publishers, New Delhi, India, pp 381-391.

Vedavathy S, Sudhakar A and Mrudula V 1997. Tribal medicinal plants of Chittoor. Ancient Science of Life **16(4):** 307-331.

Venkatachalam S 2003. Assessment of microbial diversity and fertility status of shola soils of Nilgiris. MSc Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, 152p.

Vennila S, Kalaiselvi T, Fernandaz CC and Sivagamy K 2023. Dynamics of microbial communities in bamboo rhizosphere soil under natural ecosystem. Agriculture Association of Textile Chemical and Critical Reviews Journal **11(3):** 335-340.

Wall DH and Virginia RA 1999. Controls on soil biodiversity: insights from extreme environments. Applied Soil Ecology **13(2):** 137-150.

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