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Seasonal changes in microbial community structure and nutrients content in rhizospheric soil of *Aegle marmelos* tree

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Abstract. A preliminary investigation was carried out on dominance of different types of microbial communities at different monsoon seasons in rhizospheric soils of *Aegle marmelos* tree. Nutrients content of soil were also determined simultaneously to correlate with the microbial population. Results show that the rhizosphere of *Aegle marmelos* contains gram-negative bacteria, *Rhizobium*, *Azotobacter*, Actinomycetes and Yeast and major plant nutrients and their count as well as dominance changes with moisture content in rhizosphere. Except actinomycetes all the microorganisms were found highest during monsoon season whereas in post-monsoon season Actinomycetes were dominant. Amount of water in rhizosphere soil also affects soil chemical properties. Soil pH, organic carbon, C:N ratio, available nitrogen and available phosphorus were recorded maximum in monsoon, whereas electrical conductivity and total nitrogen content were found maximum in post-monsoon.
Keywords: Microbial diversity, microbial count, nutrients content, *Aegle marmelos.*

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Introduction

Bael tree (*Aegle marmelos*) is found throughout India and other cotenants in dry forest as well as cultivated. It belongs to family Rutaceae. A decoction of the bark of "Bel" and "Jambhul" is given as remedy for menstrual irregularities (Daniel et al. 2007). The roots are sweet, astringent, bitter and febrifuge. They are useful

in diarrhea, dysentery, dyspepsia, stomachalgia, cardiopalmus, vitiated conditions of *vata*, seminal weakness, uropathy, vomiting, intermittent fever, swelling and gastric irritability in infants. The leaves are astringent, laxative, febrifuge and expectorant, and are useful in ophthalmia, deafness, inflammations, catarrh, diabetes and asthmatic complaints. The unripe fruits are bitter, acrid, sour, astringent, digestive and stomachic, and are useful in diarrhea, dysentery and stomachalgia. The ripe fruits are astringent, sweet, aromatic, cooling, febrifuge, laxative and tonic, and are good for the heart and brain and in dyspepsia (Warrier et al. 2002). Plant properties affect rhizosphere ecology (Brown 1976) through root exudates. Microorganisms and nutrients content are the main components of rhizosphere. Microorganisms influence the composition and quantity of various root exudates components through their effect on root cell leakage, cell metabolism and plant nutrition. Based on differences in root zones, rhizosphere microbial communities can vary in structure and species composition (De Leij et al. 1994, Mahaffee & Kloepper 1997, Griffiths et al. 1998, Lupwayi et al. 1998). Root growth and exudates are also modified by the soil chemistry (Rengel & Marschner 2005, Nelson & Mele 2006). This increased or altered root exudates can selectively favour certain groups of microorganisms (Ikeda et al. 1997) and could alter the nutrient preference of the community of soil microorganisms (Grayston et al. 1998). Several factors such as soil amendments, soil type, its moisture, pH and temperature are known to influence the rhizosphere microflora by their effect on microbial growth and activity. However, as soil chemical and physical factor also bring impacts to bear on soil microbial communities, it would be considered pertinent to couple microbial community analysis with soil pH, nutrients status, C:N ratio etc. for maximum effectiveness (Nielsen & Winding 2002, Bending et al. 2004, Dela Iglesia et al. 2005). Nielsen and Winding (2002) and Bending et al. (2004) also reported that for maximum effectiveness, soil microbial community analysis should be coupled with a range of chemical, biochemical and physical soil measurements.

The purpose of the study was to find out the effect of root exudates of *Aegle marmelos* on qualitative and quantitative composition of microbial population along with soil chemical properties at different soil moisture level before rains, during rains and after rains. This study may help in agricultural practices to manage the chemical fertilizers according to the season and also indicated the potential role of microbial community in biofertilizers.

Materials and methods

To increase reliability of results, soil of three voluntarily established trees, grown nearby, was collected from 10 cm depth in rhizospheric zone by scrapping the soil from roots using sterilized spatula and put in UV-sterilized plastic bags. Samples were collected three times i.e. during pre-monsoon, monsoon and post-monsoon seasons from the same locations. Samples were brought to laboratory within two hours and stored at 4°C till analysis. For microbial analysis 10 g sample of each soil from three replicated sites was emulsified in 90 mL sterilized water in aseptic conditions. Now serial decimal dilutions were made from this suspension up to $1:10^{-10}$. One mL of each dilution (100 µL) was used to inoculate plates in triplicate containing specific growth media for different microorganism like Nutrient agar (for total viable count), MacConkey agar (for gram-negative bacteria), congo-red yeast extract mannitol agar (for *Rhizobium*), Ashby's mannitol agar (for *Azotobacter*) Kenknight and Munaiers medium (for *Actinomycetes*) and yeast extract agar (for Yeast) (Subba Rao, 2001). For chemical analysis the soil samples were air dried and sieved to pass through a 2 mm sieve. The pH and electrical conductivity (EC) were determined using a 1:2.5 (w:v) soil:

water ratio and Systronic pH meter and conductivity meter, respectively. Organic carbon was determined by wet oxidation method outlined by Walkley and Black (1934). The available nitrogen and phosphorus were estimated using standard laboratory methods (Singh et al. 2001). For total N estimation, Kjeldahel method was followed as outlined in AOAC (1970). Average values of microbial count and nutrients content for the three replicates are presented here.

Results

Data revealed that number of all the microorganisms studied increased in monsoon compared to pre-monsoon (Table 1). During post-monsoon number of gram-negative bacteria, *Rhizobium* and yeast decreased compared to both the seasons and varied between 1.564 to 3508.7, 10.07 to 1153.53 and 36.56 to 4793.52 x 10^5 cfu g^{-1} soil. Number of total viable microorganisms and *Azotobacter* also decreased in post-monsoon compared to monsoon and varied between 267.08 to 3815.8 and 6.11 to 150.54 x 10^5 cfu g^{-1} soil. On the other hand number of actinomycetes increased in post-monsoon than monsoon and varied between 0.0098 to 0.0104 x 10^5 cfu g^{-1} soil. Number of *Actinomycetes* was also found maximum in post-monsoon. Soil pH, organic carbon and available phosphorus contents follows the same trend and recorded maximum in monsoon followed by post-monsoon and minimum in pre-monsoon seasons and varied between 7.33 to 8.19, 0.79 to 1.15% and 0.017 to 0.049%, respectively (Table 2). The content of C:N ratio and available nitrogen in soil were found minimum in post-monsoon and maximum in monsoon and varied between 0.67 to 6.91 and 0.05 to 0.19%, respectively (Table 2). Electrical conductivity

Table 1 Effect of seasons on microbial count (x 10^5).

Microorganisms	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
Total Viable Count	267.08	3815.8000 1328.7100*	1035.4257 287.6800** (-) 72.8600***
Gram Negative Bacteria	11.12	3508.7000 31453.0600*	1.5640 (-) 85.9400** (-) 99.9600***
Rhizobium	11.12	1153.5300 10273.4700*	10.0700 (-) 9.4400** (-) 99.1300***
Azotobacter	-	150.5400 -	6.1100 - (-) 95.9400***
Actinomycetes	-	0.0098 -	0.0104 - 6.1200***
Yeast	567.29	4793.5200 744.9900*	36.5600 (-) 93.5600** (-) 99.2400***

Note: * per cent increase in microbial count during mon-soon over pre-monsoon season; ** per cent increase in microbial count during post-monsoon over pre-monsoon season; *** per cent increase in microbial count during post-monsoon over monsoon season

Table 2 Effect of seasons on nutrients content of soil.

Nutrients	Seasons		
	Pre-monsoon	Monsoon	Post-monsoon
pH (1:2.5 soil: water ratio)	7.3300	8.0019	7.8000
		11.0073*	6.4100**
			(-) 4.7600***
EC ($mScm^{-1}$)	0.1930	0.1170	0.3280
		(-) 39.3800*	69.9500**
			180.3400***
Organic Carbon (%)	0.7940	1.1518	0.8751
		41.2800*	10.2100**
			(-) 21.9900***
Total Nitrogen (%)	0.5900	0.3800	1.3500
		(-) 35.0059*	128.8100**
			255.2600***
C:N Ratio	2.2811	6.9095	0.6761
		202.9000*	(-) 70.3600**
			(-) 90.2100***
Available Nitrogen (%)	0.0700	0.1900	0.0500
		171.4300*	(-) 28.5700**
			(-) 73.6800***
Available Phosphorus (%)	0.0170	0.0049	0.0420
		188.2400*	147.0600**
			(-) 14.2900***

Note: * - per cent increase in nutrients content mon-soon over pre-monsoon season; ** - per cent increase in nutrients content during post-monsoon over pre-monsoon season; *** - per cent increase in nutrients content during post-monsoon over monsoon season

and total nitrogen content of rhizosphere soil ranged between 0.117 to 0.328 $mScm^{-1}$ and 0.38 to 1.35%, respectively and were found maximum in post-monsoon followed by pre-monsoon and monsoon season (Table 2).

Discussion

Favorable environmental conditions in microclimate of rhizosphere during monsoon may result in the highest number of all the microorganisms except actinomycetes in this period. Preferential increase in gram-negative bacteria in root zone of many plants over *Azotobacter* (Subba Rao 2001) and actinomycetes (Gandhi 1968) were also reported. Root exudates neutralizing the soil pH and alters the microclimate of the rhizosphere through liberation of water and carbon dioxide (Subba Rao 2001). Higher moisture content during monsoon further enhances soil pH of rhizosphere due to dilution effect. During monsoon period heavy rains occurred in Gujarat. When rain water infiltrate and percolate through soil layers in rhizosphere it left the plant and animal debris brings with from surface area in the rhizosphere which may contribute in organic carbon content. Large effect of seasonal changes in soil moisture, soil temperature and carbon input on soil microbial biomass and its activity was also reported by Ross (1987) which in turn, affect the ability of soil to supply nutrients to plants through soil organic matter turnover (Bonde & Roswall 1987). Microbial biomass has been reported to vary seasonally (Patra et al. 1990). Singh et al. (1989) have also reported a seasonal variation in the microbial C, N and P in forest and

savanna. Reduced number of total viable microorganisms, gram-negative bacteria, *Rhizobium*, *Azotobacter* and yeast in post-monsoon than monsoon (Table 1) reduces the utilization of carbon for their activity and thus indirectly contributes to increased organic carbon. Lower soil temperature during monsoon and post-monsoon seasons resulted in lower oxidation of organic matter hence organic carbon content increased in these seasons compared to pre-monsoon. Effect of climatic conditions on soil organic carbon was also reported by Jenny (1980).

Better root growth and higher urease activity of root exudates in monsoon may result in higher available nitrogen in monsoon. The root exudation in cold environment during monsoon season may also contribute to nitrogen input. Huge input of nitrogen into soil by decay of root exudation in cold temperate deciduous forest was reported by Vogt et al. (1986).

Although the favorable microclimate during monsoon and post-monsoon seasons in rhizosphere resulted in increased microbial number and activities and they stimulate the exudation of organic substances from roots but they also consume a substantial part of them (Sundin et al. 1990). It may also be a reason to non-consistent nutrient accumulation in soil.

It can be concluded that the rhizospheric soil of *Aegle marmelos* tree contains all the major agriculturally important microorganisms, their succession and dominance can be correlated with soil moisture content and its chemical properties.

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