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Deciphering Rhizospheric Bacterial Diversity Associated with three Threatened Medicinal Plants of Amarkantak Region in Central India

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This study investigates the rhizosphere effect exerted on the microbial communities by three rare endemic and threatened medicinal plant species of the Amarkantak region of Central India. Amarkantak region with its rich and varied biodiversity is natural habitat for various tribal communities. These tribal inhabitants use different plants for their basic necessities which have led to its indiscriminate exploitation. Some of these selected threatened and rare medicinal plants *viz*., *Litsea glutinosa, Rubia cordifolia* and *Pueraria tuberosa* of Amarkantak region require efforts for conservation and its propagation. A microbial community is considered to be an important factor for improving soil quality. Structure of soil bacterial communities not only differs in different habitats but also between rhizosphere and non-rhizosphere. Microbial population associated with these medicinal plants was examined with respect to colony forming unit and the rhizosphere:soil ratio. A positive R:S ratio was found and the total viable count of all the soil samples from different site range from 10 x 10^3 to 132 x 10^3. The R:S ratio was found to range between 1.061 and 3.55 indicating positive rhizospheric effect. Out of 79 bacterial isolates, over seventy five percent of the isolates were found to be Gram negative rods.

Key words: R:S ratio, Rhizosphere effect, Threatened Medicinal Plant, Tribal Community.

Amarkantak is situated in Anuppur district of Madhya Pradesh in India. Amarkantak with a good forest cover has been notified as Achanakmar-Amarkantak biosphere region because of its rich and varied biodiversity[1,2]. This region provides a natural, invaluable store house of indigenous medicinal plant diversity that is of great importance for mankind[3]. Different tribal communities are the native inhabitants of these forests. One of the most primitive types found in this region is Baiga which are still found residing in forest villages away from general population[4]. Approximately 60-80% of the world's population still relies on traditional medicines for the treatment of common illnesses[5]. Vast population of India relies on traditional plant medicines by various rural and tribal communities through different traditional practices[6]. Traditional knowledge of these tribals is based on years of experience and provides guidance and solution for not only sustainable environment but also use of medicinal plants for the treatment of various diseases[7].

Unsustainable harvesting of these medicinal plants has led to exploitation and decrease of the species. Systematic efforts to exploit the valuable potential are still lacking.[8] The large scale deforestation of green forest wealth, a renewable resource, is leading to an accelerated

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loss of valuable or potentially valuable biodiversity, extinction of species and genetic erosion. It has been reported by Botanical Survey of India that around 93% of medicinal plants of India now belong to endangered species. Some of the important medicinal plants in this area are on the verge of extinction or their population is threateningly low[7]. The root surface and surrounding rhizosphere are significant carbon sinks[9]. Photosynthate allocation to this zone can be as high as 40%[10]. Thus, along root surfaces there are various suitable nutrient rich niches attracting a great diversity of microorganisms, including phytopathogens. Root exudates provide a lot of nutrients for the soil microbes and energy materials. The types and quantities of rhizo-microbes are observed to be higher than the non-rhizosphere soil and also its metabolic activity is higher than the non-rhizosphere microorganisms[11]. Plant associated microorganisms fulfill important functions for plant growth and health. These rhizospheric microorganisms could be exploited for its innumerable properties and active metabolites[12]. Direct plant growth promotion by microbes is based on improved nutrient acquisition and hormonal stimulation.

Previous studies have revealed that different rhizosphere microbial communities are associated with different plants[13]. The divergence of microbial population and diversity in the rhizosperes of different plants may be due to variations in the quantity and quality of compounds released by different plants[14]. The magnitude of the rhizosphere effect depends mainly on the nature and amount of root exudates which appear to be related to plant age as well as species on one hand and edaphic and climatic factors on the other. The influence of individual plants is reflected in the rhizosphere as the R:S (rhizosphere to non rhizosphere ratio). With this background, the present study was carried out to investigate the rhizosphere effect in three threatened and rare medicinal plants species collected from Amarkantak region of Indian Central.

MATERIALS AND METHOD

Study Area

Amarkantak is situated at the border of Madhya Pradesh and Chhattisgarh. It is located at 22.67°N latitude and 81.75°E longitude. The climate of the Amarkantak is humid and mesothermal with abundant rainfall throughout the year. Amarkantak is an ideal plateau for dense vegetation cover because of its climate and rainfall and the hills and forest of Amarkantak are the treasures having valuable herbs and medicinal plants[16]. The mean temperature ranges from 21°C to 31°C. May and June are the hottest months and temperature is rising to about 31° to 34°C. December and January are coldest month. During these months the temperature sometimes fall to 0°C. Total annual rainfall is over 1000m. Maximum rainfall is recorded during July to September[16]. Amarkantak area covers parts of Madhya Pradesh and Chhattisgarh, thus an interstate Biosphere Reserve, was notified as Achanakmar-Amarkantak Biosphere Reserve in March 2005 and is fourteenth such biosphere reserve. Study area and tribal community were selected with the help of the information collected by casual interaction with several herbalists, medicine men and women of different tribal hamlets.

Collection of Soil Samples

Rhizosheric and non-rhizospheric soil samples were collected from three medicinal plant species from different sites of Amarkantak region during the month of September. The soil samples in triplicates were collected from various localities of Amarkantak *viz*., Bhundakona, Jaleshwar. Jaleshwar is 8km away from Amarkantak whereas Bhundakona is 10km away from Amarkanatak. All the samplings were done in triplicates. The rhizospheric soil was collected in sterile polythene bags and brought to the laboratory and stored at the 4 ºC until further use.

Soil samples collected from each sites were also analysed for physico-chemical properties such as particle size, organic carbon, nitrogen, Ca, Mg, K, available P and texture of soil.

Isolation and Enumeration of microorganisms

Total culturable bacterial population density was determined on the basis of serial ten fold dilution method by spread plate technique in triplicates (1g soil) using appropriate dilutions[17]. The CFUs (colony forming units) were recorded after 24-48 h of incubation at 28±1ºC by using the formula,

$CFUg^{-1}$ dry soil = Average number of colonies X Dilution factor

Dry weight of soil

A numerical value for the rhizosphere: soil ratio representing microbial counts in the rhizosphere divided by the microbial counts in the non-rhizosphere soil, was determined to analyse the rhizosphere effect on the microbial population.

RESULTS

An intensive survey of the study area was carried out to document the indigenous traditional medicinal knowledge of Baigas, tribal community of Amarkantak. A questionnaire was prepared to draw the maximum possible traditional knowledge of this primitive tribe with respect to medicinal plants. Data was collected and catalogued through a combination of tools and techniques of questionnaire, focused group interview and discussion with medicine men of different tribal hamlets. A questionnaire was prepared to collect information depicting traditional knowledge of tribal communities as well as ethnobotanical studies so that closer studies of such knowledge can lead to the discovery of new relationships between modern taxonomy and primitive knowledge. The questions were so framed as to include general information as well as background information of an individual from a particular tribe. It also helped in documenting some of the traditional knowledge with respect to medicinal plants of Baiga tribes of Amarkantak. This information provided us with an insight into the plant human interaction in their practical life, cultural life as well as social life. The questionnaire has been framed with the help of proforma provided by Kapoor and Mitra, 1987 [22].

The information provided by various medicine men and women of different tribal hamlets enabled to narrow down our selection of three medicinal plants and sampling site for each three medicinal plants were selected.

The rhizosphere soil of medicinal plants indicate higher microbial population than that of their corresponding non rhizosphere soil. The maximum was found to be of the rhizosphere of *Litsea glutinosa* whereas the minimum was of non rhizosphere of *Rubia cordifolia*. It has also been observed that the pH of rhizosphere soil of *Rubia cordifolia* is lower than that of *Litsea glutinosa*. It indicates that the bacterial population decrease with decrease in soil pH.

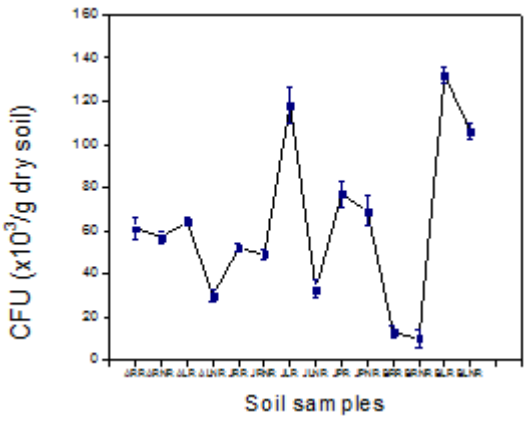
Table 1. Status of Medicinal Plants of Amarkantak region

S.No.	Botanical Name	Local Name	Status	Family
1.	*Litsea glutinosa*	Maida	Endangered	*Lauraceae*
2.	*Rubia cordifolia*	Manjistha	Vulnerable	*Rubiaceae*
3.	*Pueraria tuberosa*	Patal Kohda	Threatened	*Fabaceae*

Table 2. Soil sample analysis with respect to pH and available organic carbon, phosphate, and potassium

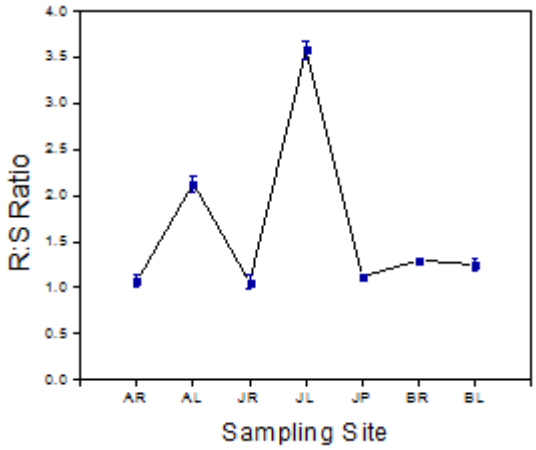
Region	Plants	pH	Available organic carbon		Available Phosphate		Available Potassium	
			% of C	F	Kg/Hect.	F	Kg/Hect.	F
Amarkantak	*Rubia Cordifolia*	5.6	4.5	High	6.3	Very Low	536.48	High
	Litsea Glutinosa	5.7	2.7	High	36	Medium	468.16	High
Jaleshwar	*Rubia Cordifolia*	5.8	5.25	High	13.5	Low	464.80	High
	Litsea Glutinosa	6.4	3.20	High	99.0	High	1178.24	High
	Pueraria Tuberosa	6.1	3.0	High	90	High	416.46	High
Bhundakona	*Rubia Cordifolia*	5.5	2.85	High	13.5	Medium	256.48	High
	Litsea Glutinosa	5.7	2.85	High	45	High	644	High

Total count and R:S ratio of three different medicinal plant species viz., *Litsea glutinosa*, *Rubia cordifolia* and *Pueraria tuberosa* from three regions that is Amarkantak, Bhundakona, and Jaleshwar are presented in figure 1 and figure 2. A positive R:S ratio was found and the total viable count of all the soil samples from different site range from 10×10^3 to 132×10^3 (Figure1).



(ARR: Amarkantak Rubia Rhizosphere; ALR: Amarkantak Litsea Rhizosphere; JRR: Jaleshwar Rubia Rhizosphere; JLR: Jaleshwar Litsea Rhizosphere; JPR: Jaleshwar Pueraria Rhizosphere; BRR: Bhundakona Rubia Rhizosphere; BLR: Bhundakona Litsea Rhizosphere; ARNR: Amarkantak Rubia Non Rhizosphere; ALNR: Amarkantak Litsea Non Rhizosphere; JRNR: Jaleshwar Rubia Non Rhizosphere; JLNR: Jaleshwar Litsea Non Rhizosphere; JPNR: Jaleshwar Pueraria Non Rhizosphere; BRNR: Bhundakona Rubia Non Rhizosphere; BLNR: Bhundakona Litsea Non Rhizosphere)
(AR: Amarkantak Rubia; AL: Amarkantak Litsea; JR: Jaleshwar Rubia; JL: Jaleshwar Litsea; JP: Jaleshwar Pueraria; BR: Bhundakona Rubia; BL: Bhundakona Litsea)

Fig. 1. CFU of microbial population of different soil samples



(ARR: Amarkantak Rubia Rhizosphere; ALR: Amarkantak Litsea Rhizosphere; JRR: Jaleshwar Rubia Rhizosphere; JLR: Jaleshwar Litsea Rhizosphere; JPR: Jaleshwar Pueraria Rhizosphere; BRR: Bhundakona Rubia Rhizosphere; BLR: Bhundakona Litsea Rhizosphere; ARNR: Amarkantak Rubia Non Rhizosphere; ALNR: Amarkantak Litsea Non Rhizosphere; JRNR: Jaleshwar Rubia Non Rhizosphere; JLNR: Jaleshwar Litsea Non Rhizosphere; JPNR: Jaleshwar Pueraria Non Rhizosphere; BRNR: Bhundakona Rubia Non Rhizosphere; BLNR: Bhundakona Litsea Non Rhizosphere)
(AR: Amarkantak Rubia; AL: Amarkantak Litsea; JR: Jaleshwar Rubia; JL: Jaleshwar Litsea; JP: Jaleshwar Pueraria; BR: Bhundakona Rubia; BL: Bhundakona Litsea)

Fig. 2. R:S ratio of isolated strains from different region

A total of 79 bacteria were isolated from fourteen soil samples of three medicinal plants. Out of 79 bacteria isolates, over seventy five percent of the isolates were found to be Gram negative. (Figure 3) In addition, majority of total bacterial isolates were found to be rod shaped.

R: S ratio determines the relative stimulation of the microorganisms in the rhizosphere of different medicinal plant species. R:S ratio of all samples indicated in the figure 2 clearly highlights that all sampling sites has positive R:S values. The R:S ratio lies in between 1.06 and 3.58. The R:S ratio was found above 1 indicating positive rhizosphere effect.

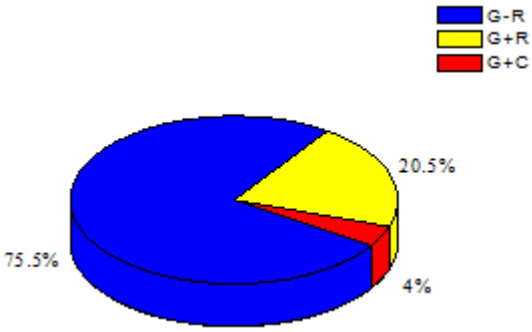


Fig. 3. Diversity of rhizospheric isolates on the basis of Gram's reaction

DISCUSSION

The indigenous knowledge of tribal communities is not only confined to use and collection of medicinal plants but also about their specific characteristics, their spatial distribution, seasonal availability and ecological relationship etc. It was observed that some of the important medicinal plants in this area are on the verge of extinction or their population is threateningly low. Hence, three medicinal plants *Litsea glutinosa*, *Rubia cordifolia* and *Pueraria tuberosa* were selected due to its endangered, vulnerable and threatened status respectively. The propagation

and mass cultivation of these plants is difficult due its nature of growing in their natural habitat. Bacteria co-existing in the rhizosphere are the most abundant microorganisms[18]. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization[19-20]. Hence, in this study rhizosphere effect in terms of total count of bacterial community and R:S ratio has been described.

The study was carried out on three different medicinal plant species *viz*., *Litsea glutinosa, Rubia cordifolia* and *Pueraria tuberosa* from three regions *viz*., Amarkantak, Bhundakona, and Jaleshwar and it was observed that the maximum cfu (132×10^3) was recorded in rhizospheric soil of *Litsea glutinosa* plant of Bhundakona and minimum cfu (10×10^3) was recorded from non rhizospheric soil of Rubia cordifolia. It was also observed that rhizospheric soil of *Litsea glutinosa* plant of Bhundakona region showed maximum count in comparison to rhizospheric soil of *Rubia cordifolia* and *Pueraria tuberosa*.

In Jaleshwar as well as Amarkantak region, the maximum R:S ratio is observed in the *Litsea glutinosa* plant whereas in Bhundakona maximum R:S ratio is recorded in the *Rubia cordifolia* plant. The microbial population of rhizosphere soil of *Litsea glutinosa* from Bhundakona region was comparatively higher than that of Amarkantak and Jaleshwar region whereas the microbial population is found minimum in the rhizosphere soil of *Rubia cordifolia* of Bhundakona region. Total viable count of all the samples also indicate that rhizosphere soil of *Rubia cordifolia* has minimum microbial population as compared to *Litsea glutinosa* and *Pueraria tuberosa*.

The rhizosphere effect is affected by many factors, like the quantity and quality of root exudates secreted by a particular plant species, in addition to prevailing edaphic and climatic conditions[15].

Though the present study focuses on the rhizosphere effect of only three medicinal plants, further investigations are needed for many such threatened medicinal plants to protect the unexplored resources for the preservation of naturally occurring medicinal and aromatic plants of such tribal areas for the future benefit of mankind. According to scientific reports, 86% of the bacterial isolates from the rhizosphere of various plants produced phytohormones, and also different vitamins. Rhizospheric bacteria produce growth promoting substances in the rhizosphere[21]. During the present study, a large number of bacteria, have also been isolated from both the rhizosphere as well as non-rhizosphere soils and further characterization of these isolated microbial diversity will be beneficial for screening potential microbes that can be exploited for biotechnological purposes.

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