Research Article

Micro flora of Rhizoshperic Soil in Some Common Medicinal Plants

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ABSTRACT
Medicinal plant plays a vital role in human health care. Medicinal plants are renewable potential natural resources. In present investigation *Spilanthus paniculata, Mentha arvensis* and *Ocimum basilicum* were selected to study diversity of root associated with microorganism. Analysis of rhizosphere and non rhizosphere soil was carried out by using selective media, It revealed that comparatively higher bacterial and fungal population was found in rhizosphere soil than non rhizosphere soil. Rhizosphere is a site of high microbial activity in and around the roots of soil. Depending on varied physicochemical properties of rhizosphere soil, it harbors a great diversity of microorganisms affecting plant growth and health. Among the bacteria dominant species was *Azatobactor Chrococcum, Pseudomonas aeruginosa, Bacillus subtilis.*

Keywords: Medicinal plant, Rhizosphere.

INTRODUCTION

-Soil is defined as the superficial layer on the earth crust, where geology and biology meet. Soil refers to the outermost loose material for plant, microorganisms and animal life, History records the rise and fall of civilization in accordance with the wise use or abuse of the soil resources. Major issue of soil resources include degradation by displacement of soil material (water and wind erosion) and the internal soil deterioration resulting from chemical degradation. (like, salinity and less of nutrients). Soil consists of mineral, organic matter, water, gases, microflora.

Microflora of soil

From Microbial point of view the soil environment is several different ways because it contain a vast number of microorganisms and is dynamic site of biological interaction in nature in which so many biochemical reactions occur for destruction of organic matter Most predominant organisms are bacteria, actinomycetes, fungi, algae, protozoa and viruses.

RHIZOSPHERE

Soil microorganisms constitute world's largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystem. The rhizosphere, a narrow zone adjacent to and influenced by living plant roots (Kennedy,1999), is a site of high microbial activity in and around roots in soil, (Sorenson,1997). It harbors a great diversity of microorganisms affecting plant growth and health. (Campbell and Greaves, 1990; Boehm *et al*.1993). The diversity and composition of bacterial taxa in the rhizosphere can be affected by several factors including plants species (Miller *et al.,* 1989), Soil type (Hoitink and Boehm, 1999), Soil management practices (Rovira *et al.,* 1990)and other environmental variables. The composition of bacterial community in the rhizosphere is important for the performance of the plants, as bacterial species can have beneficial, neutral or harmful relationships with the roots (Buchenauer, 1998; Atkison and Watson, 2000; Sylvia and chellami; 2001).

Microorganisms have been internationally introduced into soil and rhizosphere environments in attempts to enhance certain agriculturally beneficial activities such as improvement of aggregate stability (Lych, 1981), suppression of plant pathogen (Maplestone and Campbell, 1989) and promotion of plant growth (Lambert and Joos,

1989). For several decades bacteria have been introduced into soil to improve plant growth (Cooper, 1959; Mishustinand Naumova, 1962; Brown, 1974; Kloepper *et al.,* 1980; Schipper *et al.*, 1995). To date many studies on the inoculation of plant growth promoting rhizobacteria have been focused on same economically important agricultural crops, and wild flora has not been considered as research target (Glick, 1995; Bashan, 1998).

Hence the study was planned to enumerate, identify and characterize, the microorganisms present in rhizosphere soil sample of selected medicinal plants. The rhizosphere is generally taken to include the soil region intensified by microbial activities in the immediate vicinity of the root, which likely to influence the infection of root by the pathogen Rhizosphere is a site of higher microbial activity in and around the root of soil, it harbors a great diversity of microorganisms affecting plant growth and health.

In present investigation *Mentha arvensis, spilanthus paniculata, ocimum basilicum.* were selected to microbial population of root associated microorganisms. Analysis of rhizosphere soil was done.

MATERIAL AND METHODS

In present investigation, study on microbial analysis of rhizosphere soil of medicinal plants.

1) Selection of medicinal plants

Three locally available medicinal plants in Dr. Panjabrao Krishi Vidhyapeeth Akola were selected for the study and their medicinal properties are given in table.

S. No	Common name	Botanical name
1)	Pudina	*Menthe arvensis*
2)	Sabja	*Ocimum basilicum*
3)	Akalkhara	*Spilanthus paniculata.*

2) Sample collection

From the three medicinal plants 60 rhizosphere soil samples were collected by gently uprooting the plants using sterile shovel. 30 rhizosphere soil samples from each medicinal plant were taken. The plants were shaken to remove unwanted soil particles. The soil particles adhered to roots was transferred to sterile polyethylene bags. Soil adjacent few centimeters away from the root were considered as non rhizosphere soil. The samples were carried aseptically to the laboratory and were proceed within 1-2 hours.

3) Enumeration of rhizosphere soil microflora

Rhizosphere microflora of three selected medicinal plants was estimated by pour plate technique. Nutrient medium for bacteria were used. Plates were incubated at 37°C. Bacteria were counted after 24hr.

Representative colonies of bacteria, were picked and streaked on to the respective medium to obtained pure culture. The isolates were identified.

4) Identification of microorganisms

Pure culture, thus, obtained were subjected to identification based on morphological and biochemical characters.

Following properties were used for identification.

1) Colony characters
2) Gram's staining
3) By Biochemical test *viz*: Sugar fermentation. (Glucose, Lactose, Mannitol and IMViC test.).

Physiochemical analysis

A) Enumeration of pH

The pH variation was also recorded for the sample studied by mixing soil and distilled water (1:2) using pH electrode meter.

B) Enumeration of Temperature

The temperature of soil was also recorded by soil thermometer in morning.

C) Enumeration of moisture

The analysis of moisture content in soil was performed every month by taking 50gms of soil sample was freshly weighed and dried at 105^0c, then it allowed to cool and weighed again to note down the loss of weigh on drying. The moisture percentage was calculated by the following formula

$$\text{Moisture percentage} = \frac{\text{Initial weighed of sample}}{\text{Loss of weight by drying}} \times 100$$

D) Observation table- (Soil testing Report)

Samples	Organic carbon Percentage	Nitrogen $Kgha^{-1}$	Phosphorus $Kgha^{-1}$
Mentha arvensis	0.61	223	19.20
Ocimum basilicum	0.52	213	17.80
Spilanthus paniculata	0.64	230	21.90

Mechanical analysis

Sand %	Slit %	Clay %
11%	37%	52%

RESULT AND DISCUSSION

Total sixty rhizosphere soil samples of three medicinal plant from Dr. Panjabrao Krishi Vidhyapith Akola were used for biochemical analysis.

These soil samples were inoculated on Nutrient agar and thus pure culture obtained were subjected to identification on the basis of sugar Fermentation test and IMViC test as shown in table

Table 1: Sugar Fermentation Test

Name of the Bacteria	Glucose		Lactose		Mannitol	
	Acid	Gas	Acid	Gas	Acid	Gas
A. chrococchum	+ve	-ve	-ve	-ve	+ve	+ve
P. aeruginosa	+ve	-ve	-ve	-ve	+ve	-ve
S. aureus	+ve	-ve	+ve	-ve	+ve	-ve
E.coli	+ve	-ve	+ve	+ve	-ve	+ve
B. subtilis	+ve	-ve	-ve	-ve	+ve	+ve

+ve-Represents- Positive test
-ve-Represents-Negative test

Table 2: IMViC Table

Name of the Bacteria	Indol	Methyl Red	Vogas prousker	Citrate
P. aeruginosa	-ve	-ve	+ve	-ve
S. aureus	-ve	+ve	-ve	-ve
E.coli	+ve	+ve	-ve	-ve
B. subtilis	-ve	+ve	+ve	-ve
A.chrococchum	+ve	+ve	-ve	-ve

Table 3: Morphological characters of Bacteria on selective media

S. No.	Morphological Characteristics	Selective Media	Gram Characters
1	Golden Yellow Pigmented	MSA	+ve
2	Metallic sheen	EMB	-ve
3	Mucoid colony	Azatobacter isolation agar	-ve
4	Green pigmented	Psedomonas isolation agar	-ve

On the basis of result obtain in rhizospheric soil of some common medicinal plant the percentage of microorganism is calculated.

Table 4: Organisms presents in various samples

Sample code	Samples	Organisms Found				
		Azotobacter Chrococchum	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***Escherichia coli***	***Staphylococcus aureus***
	B	-ve	+ve	-ve	-ve	-ve
SP-1	A	+ve	-ve	-ve	-ve	-ve
	B	+ve	-ve	+ve	-ve	-ve
SP-2	A	-ve	+ve	+ve	-ve	-ve
	B	+ve	-ve	-ve	-ve	-ve
SP-3	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	-ve	-ve	-ve
SP-4	A	+ve	+ve	-ve	-ve	-ve
	B	+ve	+ve	-ve	-ve	-ve
SP-5	A	-ve	+ve	-ve	-ve	-ve
	B	-ve	-ve	+ve	-ve	-ve
SP-6	A	+ve	-ve	-ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-7	A	-ve	+ve	+ve	-ve	-ve
	B	+ve	-ve	+ve	-ve	-ve
SP-8	A	+ve	+ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve
SP-9	A	-ve	-ve	+ve	-ve	-ve
	B	-ve	-ve	+ve	+ve	-ve
SP-10	A	+ve	-ve	+ve	-ve	-ve
	B	+ve	+ve	-ve	-ve	-ve
SP-11	A	-ve	+ve	-ve	-ve	-ve
	B	+ve	-ve	+ve	-ve	-ve

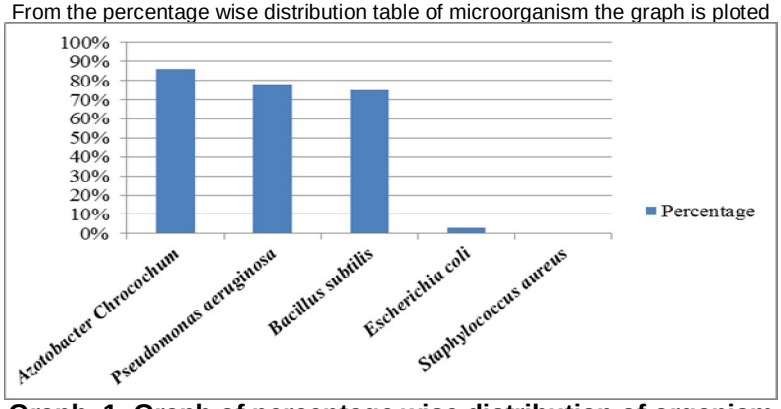
SP-12	A	+ve	-ve	+ve	-ve	-ve
	B	+ve	+ve	-ve	-ve	-ve
SP-13	A	+ve	-ve	+ve	-ve	-ve
	B	+ve	+ve	-ve	+ve	-ve
SP-14	A	+ve	+ve	-ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-15	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-16	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-17	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-18	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-19	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-20	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-21	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-22	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-23	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-24	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-25	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-26	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-27	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-28	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-29	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve
SP-30	A	+ve	+ve	+ve	-ve	-ve
	B	+ve	+ve	+ve	-ve	-ve

+ve - represent positive test.
-ve - represent negative test.

Table 5: Percentage wise distribution of organism

S. No.	Name of Bacteria	No. of positive samples	Percentage
1	*Azatobacter Chrocochum*	52	86 %
2	*Pseudomonas aeruginosa*	47	78%
3	*Bacillus subtilis*	45	75%
4	*Escherichia coli*	2	3%
5	*Staphylococcus aureus*	0	0 %

From the percentage wise distribution table of microorganism the graph is ploted



Graph. 1: Graph of percentage wise distribution of organism

Soil is mine of organism, it support the growth of diverse group of organism. The microbial diversity present in soil is greatly influence by various environmental factors. Due to difference in environmental condition the agricultural practices are greatly different in different parts of globe. This also depends on the difference in nature of soil. In our study we have to tried to focus on rhizosphere soil of medicinal plants in Dr. Panjabrao Krishi Vidhyapith Akola.

Here pH of the soil is almost alkaline (pH 9.1) (Glass electrode pH meter). In total twenty samples were analyzed. In this investigatioAn shows morphologically distinct colonies were observed. There are *Azatobacter species, Pseudomonas species, Bacillus species, Staphylococcus species, Echerichia species,*

These are subjected to species identification, *Azatobactor chrocochum* was found predominant free nitrogen fixer (86%) while *Psedommonas aeuroginosa* and *Bacillus subtilis* were predominant phosphate solubilizer (78%)whereas, *E.* coli (75%) and *S. aureus* (3%) were found in negligible percentage as compared to other.

Analysis of rhizoflora will help us to understand the nature and diversity of agro - economic microorganisms from this region. Ultimately it will help us to enhance just tried to explore the microbial diversity of rhizosphere region, there are many divert group of organism present in this region and contribute to soil fertility. In future it will be worth full to investigate the other group of microorganism like fungi, exact image of microbial diversity of rhizosphere will help to give suggestive measures for chemical and biological fertilizers.

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