

ISOLATION AND IDENTIFICATION OF RHIZOSPHERIC MICROFLORA OF SOME MEDICINAL PLANTS

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ABSTRACT :-

Plant rhizosphere is the soil nearest to the plant root system where roots release large quantity of metabolites from living root hairs or fibrous root systems. These metabolites act as chemical signals for motile bacteria to move to the root surface but also represent the main nutrient sources available to support growth and persistence in the rhizosphere. Some of the microbes that inhabit this area are bacteria that are able to colonize very efficiently the roots or the rhizosphere soil of crop plants. These bacteria are referred to as plant growth promoting rhizobacteria (PGPR). They fulfill important functions for plant growth and health by various manners. Direct plant growth promotion may result either from improved nutrient acquisition and/or from hormonal stimulation. The organisms found are P. aeruginosa, E. coli, P. vulgaris, B. subtilis, S. aureus, A. chroocochum.

Keywords : Rhizosphere, root exudation, medicinal plants.

INTRODCUTION:

The complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, as modulated by the prevalent environmental conditions (Buscot, 2005). In particular, the varied genetic and functional activities of the extensive microbial populations have a critical impact on soil functions, based on the fact that microorganisms are driving forces for fundamental metabolic processes involving specific enzyme activities (Nannipieri *et al.*, 2003).

Soil consists of following :-

Mineral :- Most dominant mineral found in soil are composed of silica, aluminium, iron while in less amount are magnesium, sodium, phosphate, sulphur, nitrogen.

Organic Matter :- The soil remain deposited on soil contribute organic substances like carbohydrates, protein, lipids. The decomposition of these results in formation of "Humus" which is dark coloured amorphous substances.

Water :- Water is retained as free water in spaces between soil and absorbed to surface of particle various component of soil are dissolved in soil water and available as nutrients for soil inhabitants.

Gases :- Gas phase of soil mainly consists of carbon dioxide, oxygen and nitrogen derived from air. These gases exists primarily in space between soil particles, which are not filled with water. Although, small amount of gases specially carbon dioxide dissolved in water.

Microflora of Soil :- From microbial point of view the soil environment is in several different ways because it contain a vast number of micro-organisms and is dynamic site of biological interaction in nature in which so many biochemical reactions occur for destruction of organic matter.

The rhizosphere effect on most commonly found microorganisms viz. bacteria, actinomycetes, fungi, algae and protozoa is being discussed here with in the following paragraphs.

RHIZOSPHERE

The term rhizosphere was first defined over a century ago by Lorentz Hiltner and redefined by Pinton as the zone that includes the soil influenced by the root along the root tissues colonized by micro-organisms.

The rhizosphere is generally taken to include the soil region intensified by microbial activities in the immediate vicinity of the root, which is likely to influence the infection of the root by the pathogen. Rhizosphere is a site of higher microbial activity in and around the root of the soil, it harbours a great diversity of micro-organisms affecting plant growth and health.

Medicinal plants play a vital role in human health care. Medicinal plants are renewable potential natural resource. In the present investigation Tulsi (Sabja) (*Ocimum basilicum*), Pudina (*Mentha arvensis*), Aloe vera (*Aloe barbadensis*), Lemon Grass (*Cymbopogon flexuosus*), Akkalkara (*Spilanthus Paniculata*), *Tinospora cardifolia* (*Menispermaceae*), *Catharanthus roseus* (*Apocynaceae*), *Vitex Nigundo* (*Lamiales*), *Muntingia* (*Muntingiaceae*), *Crinum asiaticum* (*Amaryllidaceae*) were selected to study microbial population of root associated micro-organisms. Analysis of rhizosphere soil was done.

India is of the richest plant medical cultures in the world. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considered "all" plants as potential source of medicinal substances, soil micro-organisms constitute World's largest reservoir of biological diversity and are crucial to the functioning of terrestrial ecosystem. 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 micro-organisms affecting plant growth and health.

MATERIAL AND METHODS

In the present investigation, study on microbial analysis of rhizospheric soil of medical plants.

MATERIALS :-

Material used for isolation and identification :

1. Different materials used are soil sample from Botanical Garden of Shri Shivaji College, Akola
2. Media Used – Nutrient agar, Pseudomonas Isolation Agar, E.M.B. Agar, MacConkey Agar, Mannitol Salt Agar, Mular Hinton Agar
3. Reagents and staining solution – Gram's Crystal violet, Gram's Iodine, Kovac's Indole Reagent, Safranin, Methyl Red, Tryptone broth, MR-VT medium, Koser Citrate Broth, Sugar Fermentation Broth.

METHOD:-

In the present investigation, study on microbial analysis of rhizospheric soil of medical plants.

Selection of Medical Plants

Five locally available medical plants in Botanical Garden of Shri Shivaji College of Science, Akola were selected for the study and their medicinal properties such as – Aloe vera (*Aloe Barbadensis*), Tulsi (*Ocimum Basilicum*), Gawati Chaha (*Cymbopogon Flexuosus*), Akkalkara (*Spilanthus Paniculata*), *Tinospora* (*Tinospora Cordifolia*), *Catharanthus Roseus* (*Catharanthus Roseus*).

Sample Collection :

From the five medical plants 15 Rhizospheric soil samples were collected by gently uprooting the plants using sterile shovel. 10 Rhizospheric soil samples from each medical plant were taken. The plants were shaken to remove unwanted soil particles. The soil particles adhered to roots were transferred to sterile polyethylene bags. Soil adjacent few centimeter away from the roots were considered as non rhizospheric soil. The samples were carried aseptically to the laboratory and were processed within 1-2 hours.

Media :

The media used for isolation of rhizospheric bacteria were Nutrient agar, Nutrient broth, Eosine Methylene Blue Agar, Pseudomonas Isolation Agar, Mannitol Salt Agar, MacConkey Agar, Azotobacter Isolation Agar, and Mueller-Hinton Agar which were obtained from Himedia, India.

Enumeration of rhizospheric soil microflora :

Rhizospheric microflora of five 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 24 hours.

Representative colonies of bacteria were picked and streaked onto the respective medium to obtain pure culture. The isolates were identified.

Identification of Microorganisms:

Gram Staining and microscopic study was performed for the isolation of rhizospheric microorganisms from Nutrient agar plates. The Biochemical test performed by Indole Test, Methyl Red, Vogus Proskauer, Enzyme test, Amylaze, Gilitinase, Oxidase and catalase test were performed. Identifications of isolated obtained in pure cullture was based on gram staining, morphology, growth characteristics on selective and differential media and biochemical tests.

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 weighted and dried at 105°C, then it allowed to cool and weighted again to note down the loss of weigh on drying.

D. Antibacterial Activity of Medicinal Plants:-

The antibacterial activity of the rhizospheric bacteria was tested by agar cup diffusion method. Briefly 20ml of Mueller Hinton Agar was poured into the Petri-dish and 8 mm well bored in agar.100µL of extracts (1500µg/ml) was poured into the wells. The plates were incubated for 24 h at 37°C and the zone of inhibition was measured in mm. Antibacterial activity was further characterized by determining whether bacteriostatic r bactericidal. The test was performed by swabbing of the growth inhibition zone of the plate and then the swab was streak onto Mueller Hinton Agar plate and incubated aerobically at 37°C for 48 hours. The presence of growth in Mueller Hinton Agar plate was interpreted as an inhibitory activity i.e. bacteriostatic, while no growth was interpreted as bactericidal.

Table 1: Colony Character of Rhizospheric bacteria

Sr. No.	Name of Isolate	Size	Shape	Color	Margin	Elevation	Opacity	Morphology
1	<i>Pseudomonas auruginosa</i>	1-2	Roughly Circular	Bluish Green	Flat	Irregular	Opaque	Gm-ve rod Non Capsulated
2	<i>Staphylo-coccus aureus</i>	2-4	Circular	Golden Yellow	Smooth	Convex	Opaque	Gm+ve Cocci arranged in cluster
3	<i>Bacillus subtilis</i>	1-2	Circular	Orange	Smooth	Convex	Opaque	Gm+ve Cocci
4	<i>Azotobacter chrochum</i>	1-2 mm	Circular	Yellow	Smooth	Convex	Translucent	Gm-ve short rod
5	<i>Escherichia coli</i>	3 -4 mm	Circular	Green metallic sheen	Entire	Flat	Opaque	Gm-ve short rod

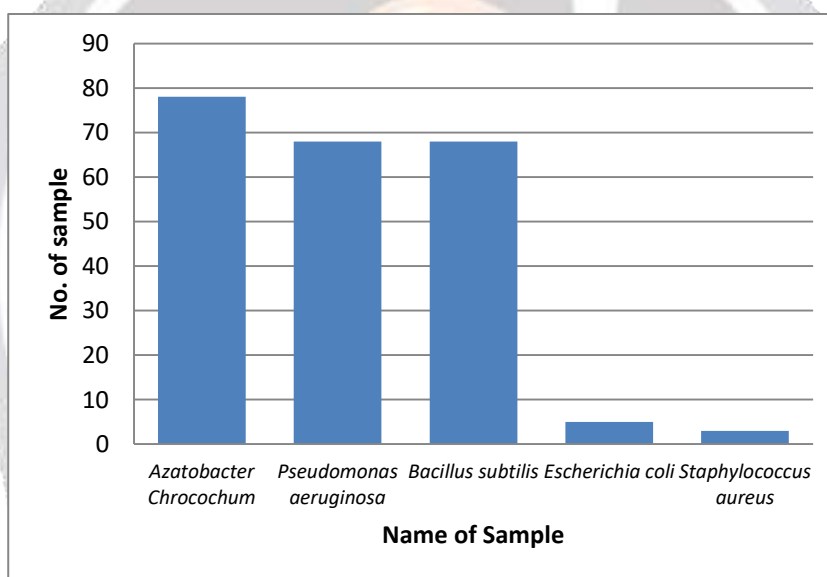
RESULTS AND DISCUSSION:

Total fifteen rhizospheric soil samples of five medical plants from botanical garden of Shri Shivaji College of Arts, Commerce & Science, 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 were performed.

Table 2: Percentage wise distribution of organisms

Sr No.	Name of Bacteria	Name of Positive Samples	Percentage
1	<i>Azotobacter Chrocochum</i>	78	78%
2	<i>Pseudomonas aeruginosa</i>	68	68%
3	<i>Bacillus subtilis</i>	68	68%
4	<i>Escherichia coli</i>	5	5%
5	<i>Staphylococcus aureus</i>	3	3%

Fig.1: Percentage wise distribution of organisms.

From the above observations predominant bacteria in rhizosphere area of saline soil were *Azotobacter chrocochum*, *Pseudomonas aeruginosa*, *Bacillus subtilis*.

From percentage wise distribution of micro-organisms the graph was plotted.

Antibacterial Activity of Medicinal Plants:-

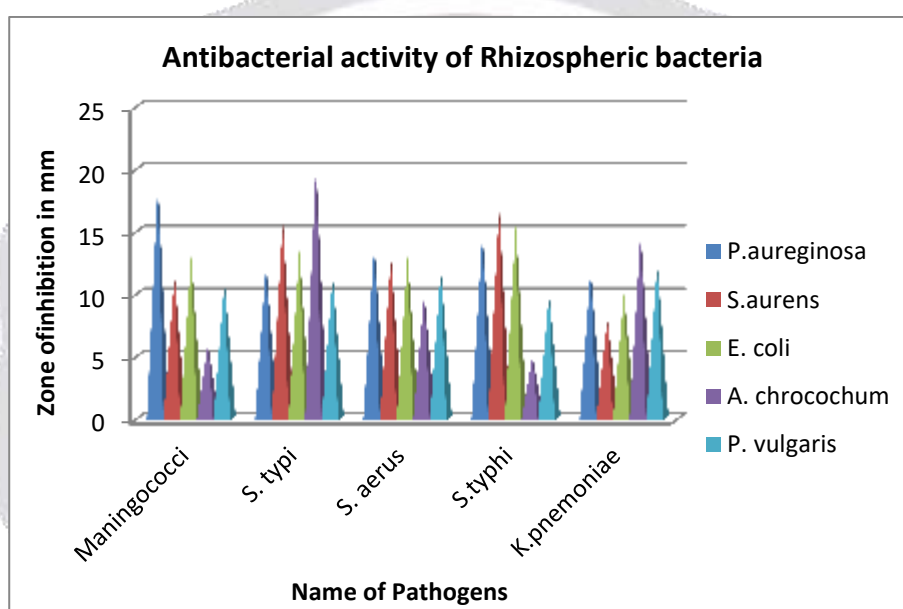
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OBSERVATION:

Table 3: Antibacterial activity of the rhizospheric bacteria from isolated from rhizospheric soil.

Name of isolates	<i>Meningococci</i>	<i>S. typhi</i>	<i>S. aerus</i>	<i>S.typhi</i>	<i>K.pneumoniae</i>
<i>P.aeruginosa</i>	19 mm	12.5 mm	14 mm	15 mm	12 mm
<i>S.aureus</i>	11.5 mm	16 mm	13 mm	17 mm	8 mm
<i>E. coli</i>	13 mm	13.5 mm	13 mm	15.5 mm	10 mm
<i>A. chrocochum</i>	6 mm	20.5 mm	10 mm	5 mm	15 mm
<i>B. subtilis</i>	11 mm	11.5 mm	12 mm	10 mm	12.5 mm

Fig. 2:- Antibacterial activity of the rhizospheric bacteria from isolated from rhizospheric soil.



DISCUSSION :

The rhizosphere, is defined as the soil zone under the influence of plant roots, a site of high microbial activity, characterized by a great ray of complex and dynamic physical, chemical and biological interactions. In the rhizosphere, microorganisms have an important role in the organic matter transformations and biogeochemical cycles of plant nutrients.

Pseudomonas aeruginosa was isolated by using soil sample. *Pseudomonas aeruginosa* was showing Gram negative, *Cocobacilli* motile bacteria on nutrient agar green pigmented colony was found, on *Pseudomonas* isolation agar yellowish greenish flurescent colony was found, we have found in sugar fermentation on acid and gas production for isolation of *S. aureus* soil sample were used. *S. aureus* were showing Gram +ve cocci, non-motile bacteria on nutrient agar yellow colour colonies was found on Mannitol Salt agar golden yellow colour colony was found. We have found in sugar fermentation on acid and gas production.

Azotobacter chrocochum commonly obtained from soil sample. *Azaotobacter chrocochum* was showing Gram –ve cocobacilli. On nutrient agar or *Azotobacter* isolation agar Mucoid colony was found. As per biochemical test Glucose, Lactose, Mannitol.

Escherichia coli was isolated by using soil sample. *Escherichia coli* was showing Gram –ve cocobacilli on E.M.B. green metallic sheet colony was found. As per Biochemical Indol, MRVP, Citrate.

Bacillus subtilis was isolated by using soil sample. *B. subtilis* was showing Gram +ve, rod shaped on Nutrient agar and on MacConkey agar white colour colonies was found. As per biochemical

characteristics we have found in sugar fermentation test Glucose, Lactose, Mannitol. In enzyme test this isolate were showing Gelatinase +ve, Amylase –ve.

Staphylococcus aureus was isolated by using soil sample. *S. aureus* was showing Gram +ve, rod shaped on nutrient agar and on Mannitol Salt Agar golden yellow pigmented colonies was found. Asper biochemical characteristics we have found in sugar fermentation test Glucose, Lactose, Mannitol.

These are subjected to species identification, *Azotobacter chroocochum* was found predominant free nitrogen fixer (78%) while *Pseudomonas aeruginosa* and *Bacillus subtilis* were predominant phosphate solubilizer (68%), whereas *E. coli* (5%) and *S. aureus* (3%) were found in negligible percentage as compared to other.

Azotobacter was found to be more significantly effective against all tested pathogen such as *K. pneumoniae* produced (20.5 mm) diameter for zone of inhibition, *S. aureus* (5mm), *Salmonella* (10 mm), *S. typhi* (15 mm) and *Manigococci* (6 mm). In the present study, bacterial pathogen *K. pneumoniae* was found to be highly inhibited by the rhizospheric microflora of medicinal plant extract of *Azotobacter* with the zone of inhibition 20.5 mm. The pathogen like *Manigococci* and *Salmonella* were more effectively inhibited by the rhizospheric microflora of medicinal plant extract. This study similar with S. L. Sukanya *et al.*, (2009).

The study was assess the antibacterial effect of some medicinal plant extracts and their synergistic antibiotic and non-antibiotic drugs against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extract of medicinal plants were prepared using Soxhlet apparatus for alcoholic extract, and water reflux for aqueous extracts. The results of this study showed that there is a decrease in MIC in case of methanolic extract of *E. camaldulensis* against *E. coli* (3.125 mg/ml), and the methanol and aquatic extract of *F. sycomorus* (leaves) against *S.aureus* varying from 6.25 to 3.125 mg/ml, and the ethanol extract of *E. camaldulensis* against *P. areuginosa* (6.25 mg/ml). Thereby, our results indicate the possibility of using these extracts in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo. Mohamed Jouda *et al.*, (2013).

To evaluate the antibacterial activities of some traditional medicinal plants on several pathogenic bacteria, which can cause diseases in human. Thirty four medicinal plants belonging to twenty-four families were selected based on medicinal reports practiced by the indigenous people and screened for their antibacterial activity against eight human pathogenic bacteria (*Bacillus subtilis*, *B. megaterium*, *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi* and *Shigella dysenteriae*) by disc diffusion and agar cup methods. Among them *Psidium guajava*, *Terminalia arjuna*, *Phyllanthus embelica*, *Terminalia chebula*, *Justicia adhatoda*, and *Ocimum sanctum* showed significant antibacterial activity against the human pathogenic bacteria. The largest zones of inhibition (22 mm in diameter) were recorded against *S. dysenteriae* and *B. cereus* with the fruit extracts of *O. sactum*. Uddin *et al.*, (2013)

Soil provides a very good environment for the proper growth of microbes such as protozoa, viruses, fungi & bacteria. Some micro-organisms were able to colonize soil surrounding plant roots and were called 'the rhizosphere micro-organisms' or rhizoflora. Rhizobacteria had the ability to multiply and colonize plant root at all stages of plant growth, in the presence of a competing microflora. The activities between rhizobacteria of a leguminous plant as well as that of a non-leguminous plant treated were also compared. The isolated strains were also screened for ammonification, nitrification and phosphate solubilization activities. Microbial activity in soil was measured by soil respiration method. To assess the rhizosphere effect R: S ratio was also estimated. The overall results show that the biofertilizers support more microbial growth than the chemical fertilizers. These results support the application of biofertilizers instead of chemical fertilizers. Sandra Yesudasan Miranda *et al.*, (2015).

CONCLUSION :

This study deals with the isolation identifiable and antimicrobial activity of rhizospheric bacteria microflora of some medicinal plants. From various sources at suitable, pH, temperature, Nitrogen source. We found phosphate solubilizer and nitrogen fixer.

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 microorganisms like fungi, exact image of microbial diversity of rhizosphere will help to give suggestive measures for chemical and biological fertilizers.

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