Studies on potential of rhizobacteria associated with medicinal plant roots for bio control of common crop diseases

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ABSTRACT

Rhizosphere is the area of intense microbiological activity. Plant growth promoting rhizomicroflora inhabits rhizosphere of plants and enhances plant growth through production and release of metabolites and also inhibit soil borne plant pathogens by the production of substances like $NH_{3,\ HCN}$ & siderophore.

The present study was intended to isolate plant growth promoting microorganisms from the rhizosphere of 6 different medicinal plants viz. *Aegle marmelos, Calotropis procera, Azadirachta indica, Withania somnifera, Lawsonia alba* and *Curcuma longa* . Total 41 bacterial and 19 actinomycetal isolates were obtained from the rhizosphere of 6 medicinal plants. All the isolates were characterized on the basis of morphological, cultural and biochemical characters. All the isolates were screened for plant growth promoting activities namely NH_3, HCN and siderophore production and also tested in vitro for antimicrobial activity against plant pathogens. The results showed that 100 % bacterial isolates except 1 isolate from *Aegle marmelos* produced NH3, more than 50% isolates produced HCN and siderophore production was shown by 66.6 % isolates from *Aegle marmelos* and *Calotropis procera*. 25-50 % isolates from remaining plants showed siderophore production. Results of antimicrobial activity against plant pathogens showed significant results with the isolates obtained from *Calotropis procera* and *Aegle marmelos.*

Among 19 actinomycetal isolates 94.21% isolates produced NH_3, 63.1% isolates produced HCN and siderophore. Results of antimicrobial activity against plant pathogens showed significant results with the isolates obtained from *Withania somnifera*. Thus these isolates can be used as sources for plant growth promoting substances and biocontrolling agents and hence are agriculturally important.

Key words- rhizosphere, medicinal plants, PGPR, antimicrobial activity

INTRODUCTION:

Many plant diseases are soil borne. Plant pathogens enter through roots. Control of plant diseases is essential to sustain a reasonable amount of production of every crop. In intensive agricultural practices one of the methods to control soil borne plant diseases biological control has received attention. Interest in biological control has increased recently and fuelled by public concerns over the use of chemicals. This is because control by chemicals is expensive and harmful. It causes health hazards to the farmers, & makes pathogens resistant and also kills harmless and useful organisms.

Rhizosphere is the area of intense microbiological activity that extends several millimeters from the root system of the growing plants (*Hiltner,1904*) Rhizosphere microorganisms differ from one plant to other. The population of microorganisms in rhizospheric and non rhizospheric soil differs qualitatively and quantitatively (*Atlas and Bartha1987*). The plants alter rhizosphere population through root exudation and the sloughing off root cells. The root exudates constitute organic and inorganic compounds (*Rovira, 1965*). The organic exudates contain carbohydrates, organic acids, nucleotides, flavonones, enzymes etc. Sugars such as glucose, fructose, sucrose, xylose, maltose, rhamnose, arabinose, raffinose and few oligosaccharides. The amino acids in root exudates are leucine, valine, glutamine, aspargine, serine, glycine, glutamic acid, phenyl alanine, threonine, tyrosine, lysine, proline, tryptophan etc. Vitamins found in root exudates are biotin, thiamin, pantothenate, niacin, choline, inocitol, pyridoxine, para- amino benzoic acid, meta methyl nicotinic acid. The nucleotides, flavono nes and enzymes are leaked from the zone of active cell division immediately behind the root cap. (Dubey R.C. & Maheshwari D.K.). The Rhizosphere region is a highly favorable habitat for the proliferation and metabolism of numerous types of micro organisms (*Alexander, 1977*). Rhizosphere contains varied number of bacteria, fungi and actinomycetes as important organisms.

Rhizospheric bacteria are known to influence plant growth by direct and indirect mechanisms. These bacteria are also called as plant growth promoting rhizobacteria. These organisms enhance the growth of plants by producing phyto

hormones like Indole acetic acid, by phosphate solubilization or by nitrogen fixation. Plant growth promoting bacteria control the plant pathogens through production of antibiotics, lytic enzymes, HCN production, and siderophore production. India is the richest source of medicinal plants. The different parts of medicinal plants extracts have medicinal uses (Jain medicinal plants).

Rhizosphere is characterized by greater microbiological activity than the soil away from the plant roots. The intensity of such activity depends on the distance to which exudations from the root system can migrate. The term 'rhizosphere effect 'indicates the overall influence of plant roots on soil micro organisms. It is now clearly established that greater number of bacteria, fungi and actinomycetes are present in the rhizosphere soil than in the non rhizosphere soil (subbarao).Several factors such as soil type, its moisture, pH and temperature and age & condition of plants are known to influence the rhizospheric effect. (Subbarao N.S.)

The Rhizosphere of medicinal plants may harbor some interesting organisms because roots of some medicinal plants may be used as medicine and exudates of such plants may contain different compounds. Root exudates influence the proliferation and survival of root infecting pathogens in soil either through fungistasis or inhibition of pathogens in the rhizosphere (Subbarao 4th edition).

There are not less than 2000 wild plant species among these plants about 4865 species are categorized as medicinal plants. It is seen from the literature that reports of rhizospheric microorganisms are scanty. Many researchers used extracts of different medicinal plants like *Azadirachta indica, Aegel marmelos, Calotropis procera and Withania somnifera.* But there are very few reports about rhizospheric bacteria of above plants. In literature there is no report about PGPR and antimicrobial activity of microorganisms from rhizosphere soils of *Lawsonia alba, Curcuma longa and Withania somnifera.* Hence these plants are for study.

Medicinal plants:

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. It is evident that the

Indian people have a tremendous passion for medicinal plants and use them for a wide range of health related disturbances including simple diseases like common cold to complicated infections and for memory improvement also. People use various medicinal plants for treatment of snake bite and to enhance general body immunity.

Medicinal plants are distributed across diverse habitats and landscape elements. Around 70% of India's medicinal plants are found in tropical areas mostly in the various forest types. Less than 30% of the medicinal plants are found in the temperate region and higher altitudes and they are found to have high medicinal value. Analysis of habits of medicinal plants indicate that they are distributed across various habitats. One third are trees and an equal portion are shrubs and the remaining one third are herbs, grasses and climbers. (Shriniwas Puri)

Calotropis procera: Scientific classification

Kingdom- Plantae

Order- Gentianales

Family- Apocynaceae

Subfamily- Asclepiadoideae

Tribe - Asclepiadeae

Subtribe- Asclepiadinae

Genus- Calotropis

Species- procera, gigantean

Calotropis is a genus of flowering plants in the dogbane family Apocynaceae. They are commonly known as milkweeds because of the latex they produce. Calotropis species are considered as common weeds in some parts of the world. The plant was described as Akra in Ayurveda and used in many traditional medicines.

Calotropis procera is a wild shrub which grows upto a hight of 1-3 m and its leaves are 10-13cm wide by 17-19cm long. *Calotropis procera* is a popular medicinal plant found throughout the tropics of Asia and Africa & is used in many traditional systems of medicine.

Different parts of this plant have different effects. The milky exudation from the plant is corrosive poison. The latex is said to have mercury like effects on the human body. The latex of C.procera has been used in leprosy, eczema, inflammation, cutaneous diseases, syphilis, malarial and low hectic fevers. Latex produces a compound known as calotropin which is toxic. Calotropin is similar in structure to two cardiac glycosides.

Leaves are used in rheumatism, as an anti-inflammatory and antimicrobial. They are also used for medicinal purposes by frying in oil.

Roots as hepatoprotective agents, against cold and coughs, syphilis and elephantiasis, as an anti-inflammatory, analgesic, antimalarial and antimicrobial. Root bark has Digitalis like effect on heart. Dried latex and chloroform extract of roots has been reported to possess anti-inflammatory activity. Extracts from the flowers of *Calotropis procera* have shown strong cytotoxic activity in the patients of colorectal cancer. They are harmful to the eyes. A literature survey of *Calotropis procera* revealed that the plant contains mainly cardinolides besides steroids and triterpenes. Proceragenin is also isolated from a plant. It has antibacterial and anti aggregating activities. Besides this the plant contains many useful enzyme like protease.

Withania somnifera:

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Solanales

Family - Solanaceae

Genus - Withania

Species - somnifera

Withania somnifera also known as 'ashwagandha', Indian ginseng, in Sanskrit 'horse's smell' ,probably originating from odour of root. The species name somnifera means 'sleep inducing' in latin. It grows as short shrub (35-75 cm). A small undershrub, stem and branches covered with minute star shaped hairs, leaves ovate hairy like branches, flowers pale green small, fruit 6 mm diameter, globose, smooth, red, enclosed in the inflated membranous calyx. The root is long, tapering &light brown in colour.

The main chemical constituents are alkaloids and steroidal lactones. These include tropine & cuscohygrine. The leaves contain the steroidal lactones, withanolides, notably withaferin A which is the first withanolide to be isolated from *Withania somnifera* .

The roots of *Withania somnifera* are used to prepare the herbal remedy ashwagandha.which has been traditionally used to treat various symptoms and conditions. It is best known for its powerful adaptogenic properties, meaning that it helps mind and body adapt better to stress. It acts as an overall tonic for greater vitality and longevity. It nourishes all the bodily tissues, including joints & nerves.

Useful in carbuncles, consumption, cough, debility from old age, general weakness, promotes urination, removes functional obstruction of body and rheumatism. Three alkaloids, antibiotic and antibacterial activity has been shown in the roots and leaves.

Aegle marmelos:

Kingdom - Plantae

Division - Magnoliophyta

Class - Magnoliopsida

Order - Sapindales

Family - Rutaceae

Genus - Aegle

Species - marmelos

Common name- bael

A medium sized thorny deciduous tree grows upto 10 meters in height . leaves trifoliate, aromatic and alternate. Flowers greenish white, sweet scented & astringent. Fruit globose , pulp mucilaginous, aromatic, sweet and astringen (Shriniwas Puri). Useful parts are leaves, fruits and roots. Fruits contain marmalosin, young bark coumarin and umbelliferone, leaves contain an essential oil. Plant pacifies vitiated kapha , body pain, poison, diarrhea, dysentery, vomiting and intermittent fever. Pulp of unripe fruit is constipating where as that of ripened fruit is laxative. Leaves cure diabetes, cough, inflammation and asthma (Shriniwas Puri).

In action bael is alterative, antipyretic, ant scorbutic, digestive ,febrifuge, odoriferous, restorative, stimulant, stomachic and tonic. Useful in fever, hepatic or typhoid, bronchitis, constipation, palpitation of the heart, debility, piles, seminal weakness (A.C. Dey).

Azadirachta indica

Kingdom - Plantae

Division - Magniophyta

Class - Magnoliopsida

Order - Sapindales

Family - Meliaceae

Genus - Azadirachta

Species - indica

A large evergreen tree native to the arid regions of the Indian subcontinent. The tree is leafless only for a very short period. Leaves pinnate, flowers white and sweet scented. The plant is found throughout India. It also grows widely in several other countries of Asia, Australia, Africa and central and South America. It has been held in high esteem by Indian people for its medicinal and insecticidal properties. As a common roadside plant grown over the greater parts of India.

Neem bark is coarsely fibrous, thickness varies according to the age of the tree. External surface rough, fissured & of a rusty grey colour, inner surface yellowish. The taste is bitter and astringent. The leaves are pinnate, serrated very bitter in taste.

In action it is alterative, antihelmithic, antiarthritis, antimalarial, antiperiodic, antiseptic, appetiser, astringent, bitter tonic, constipative, contraceptive, cooling digestive, expectorant, febrifuge, flatulent, ophthalmic, purgative, resolvent, stimulant,stomachic, suppurative tonic & vermifuge.

Contains alkaloids nimbin, nimbinin, nimbidin,nimbosterin, nimbectin, fatty acids of different types. Azadirachtinoids, salanin, desacetyl salannin are also present in this tree.

It has been an age old practice to place its dried leaves between folds of cloth to ward off moths, insects and other pests in stored rice, wheat and other grains. It is now considered to have great potential as pest control agent in agriculture. It has emerged as the single most important source of pesticides and allied products. All parts of tree such as leaf , flower, fruit , seed, kernel , bark, wood and twig are biologically active.

Curcuma longa

Kingdom – Plantae

Division - Magnoliophyta

Class - Liliopsida

Subclass - Zingiberidae

Order - Zingiberales

Family - Zingiberaceae

Genus - Curcuma

Species - longa

Species name –*Curcuma longa* Linnaeus

It is a rhizomatous herbaceous perennial plant of the ginger family, Zingibereraceae., A tall herb, rootstock large, ovoid with cylindrical tubers orange coloured inside. Turmeric is of Outmost importance of Indians ceremonially,religiously and domestically.

In action turmeric is alterative, antihelminthic, antiperiodic, antiparasitic, aromatic and astringent, bitter, blood purifier, carminative, drying hot, stimulant and tonic. Useful in boils, bruises, chronic bronchitis, cold cough and coryza,eosinophilia, eyewashes, liver and skin diseases, swellings and wounds of all kinds and dental problems.

Contains curcumin, alkaloid and an essential oil.(A. C .Dey)

Rhizome improves body complexion and appetite, laxative. Useful in leprosy. Effective against bacterial infection, skin diseases ,intestinal worms, liver complaints, filarial, asthma, sprains, boils, wounds, conjunctivitis, allergic reactions against leeches. Powder of rhizome is used as antifertility agent. Useful in gastrointestinal complaints. It is digestive , diuretic and vulnerary.

Turmeric helps to regulate the female reproductive system and purifies the uterus and breast milk & in men it purifies and builds semen. It reduces fevers, diarrhoea, urinary disorders, insanity, poisoning, cough &lactation problems in general.

It helps to detoxify the liver, balance cholesterol levels, fight allergies, stimulate digestion, boost immunity & it is also an antioxidant.(agrobios june 2013)

Lawsonia alba

Kingdom - Plantae

Division - Tracheophyta

Class - Magnoliopsida

Order - Myrtales

Family - Lytheraceae

Genus - Lawsonia

Species - alba

A middle sized much branched shrub, branches 4 angled, usually ending in a sharp point. Leaves opposite short petioled , oblong, pointed at both ends. Flowers white, very fragrant. The fruit is round.

In action the drug is alterative, astringent, cephalic, prophylactic against skin diseases, refrigerant and soporific. Useful in blood dysentery, boils, burns, burning sensations of feet, enlargement of spleen, hair growth promoter. Useful in headache heart trouble, insomnia, jaundice, skin diseases, sore throat & spermatorrhoea.

Leaves contain glucoside, colouring matter, hennotanic acid.

The chief use of henna is as a pleasant orange dye for colouring palms ,nails ,feet etc. conditioning hair and as a powerful antidandruff agent.

The present project was mainly carried out to search the bacterial and actinomycetal isolates from rhizosphere soil of medicinal plants, which can be used to kill bacterial plant pathogens like *Xanthomonas citri*, *Xanthomonas oxenopodis* and fungal pathogens *Aspergillus niger, Fusarium oxysporum* which are responsible for causing many plant diseases and create a loss of crop and economic loss to the farmers. As these bio controlling agents a) Survive for longer period in the environment and remain active. b) Attack only the pathogens and not harmless

organisms c) Cause no health hazard during preparation d) No damage is caused to the environment e) Are easy to prepare on commercial scale also and apply on plants.

The search for such agents will be beneficial to the farmers and society.

Objectives of the project :-

The major objective of the project was searching for bacterial isolates from rhizosphere soil of medicinal plants which can be used as bio controlling agents to control plant pathogens in and around Solapur city.

The isolates from rhizosphere soil of medicinal plants may be able to produce antibacterial substances against some plant pathogenic bacteria. The antibacterial activity against plant pathogens can help in controlling pathogens causing severe disease to plants like oily spots of pomegranate, citrus canker, Tikka disease of ground nut, diseases caused by Fusarium oxysporum etc in and around Solapur city.

It will be an economical alternative for plant disease control against use of chemicals. The another objective of project is to isolate siderophore producing and other plant growth promoting rhizobacteria and rhizoactinomycetes from rhizospheric soil of medicinal plants and it may also help in controlling plant pathogens entering through roots.

Materials and methods:

Materials –

i) Soil samples from rhizosphere region of medicinal plants *Aegle marmelos, Calotropis procera, Azadirachta indica, Withania somnifera, Lawsonia alba* and *Curcuma longa*

ii) peptone water

iii) Sugar media viz glucose, sucrose, lactose, mannitol, maltose, xylose , arabinose, inositol and rhamnose .

iv) Glucose phosphate broth

v) Koser's citrate medium

vi) Christensen's urea agar

vii) Triple sugar iron agar

viii) Nutrient gelatin stab

ix) Starch agar

x) Milk agar

xi) Pikovskya's agar

xii) Glycine agar

xiii) Chrome Azurol S agar

xiv) Hydrogen peroxide

xv) Oxidase reagent

METHODS:

Collection of soil sample- Rhizospheric soil samples were collected from the rhizosphere of four different medicinal plants. One soil sample from each medicinal plant viz. *Aegle marmelos, Calotropis procera, Azadirachta indica, Lawsonia alba, Withania somnifera and Curcuma longa* were collected from college campus. These samples were collected at a depth of 6-10 centimeter in the rhizosphere region of medicinal plants. The soil samples were allowed to air dry at room temperature.

Enumeration:- Enumeration of bacterial population of each soil sample was studied by serial dilution and pour plate technique plating 0.1 ml of the dilution in sterile Petri plates in triplicates with nutrient agar medium. The plates were incubated at room temperature for 24-48 hours and the colony count was taken.

Isolation of organisms: - Bacteria were isolated from Rhizospheric soil samples of different medicinal plants by serial dilution technique using nutrient agar and actinomycetes were isolated using glycerol aspargine agar. Different colonies were selected cultural characters were studied colonies were transferred on nutrient agar slants.

Total 41 bacterial and 19 actinomycete isolates were obtained from rhizosphere soil samples of 6 different medicinal plants from Dayanand college campus and different regions from and Solapur city. Isolates were characterized on the basis of morphological, cultural and biochemical characters and identified upto genus level.

Actinomycetal isolates were also characterized on the basis of morphological, cultural and biochemical characters and identified upto genus level. Salt resistance, pH tolerance and antibiotic resistance pattern of isolates was studied.

Enzymatic potential: -Enzymatic potential of bacterial and actinomycetal isolates was studied.

Amylase activity :- All isolates were spot inoculated on sterile starch agar plates (peptone - 1gm, NaCl - 0.5gm, Yeast extract – 0.3 gm, starch - 2 gm, agar – 2.5gm, D/W – 100 ml, pH – 7.2) and incubated at 28 ^{0} c for 24 hours. After incubation amylase activity was examined by adding dilute iodine solution and observed for zone of clearance around the colony.

Caseinase activity :- All isolates were spot inoculated on sterile milk agar plates (nutrient agar 90ml + 10 ml skimmed milk) and incubated at 28 ^{0} c for 24 hours. After incubation caseinase activity was examined by observing zone of clearance around the colony.

Phosphate solubilization :- Pikovskaya's agar plates (glucose - 10 gm, Ca_3 $(po_4)_2$- 5gm, $(NH_4)_2So_4$ - 0.5gm, Kcl- 0.2gm, $MgSo_4$, 7 H_2O -0.1gm, MnSo4- 0.006gm, FeSo4- 0.006gm, Yeast extract – 0.5 gm, agar- 15gm, D/W -1 L, pH – 7.2) were spot inoculated with bacterial isolates and incubated at 28 ^{0} c for five days. The bacterial colonies forming clear zones were considered as phosphate solubilizing zones.

Catalase activity of all isolates was studied using hydrogen peroxide and oxidase test was performed using oxidase reagent.

Urease enzyme activity of isolates was studied using Christensen's urea agar slants by inoculating and incubating the slants at 28^0C for 24 hours. After incubation the slants were observed for colour change from yellow to magenta red.

All isolates were studied for different plant growth promoting activities like NH_3, HCN and siderophore production.

NH_3 production: - Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10ml peptone water in each tube and incubated for 48-72 hours at 28 ± 2^0C. Nesseler's reagent (o.5ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production. (Cappuccino & Sharman 1992)

HCN production: - All the isolates were screened for the production of hydrogen cyanide by adapting the method of Lorck (1948). Nutrient agar was amended with 4.4 gm glycine per liter and bacteria were streaked on modified agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5 % picric acid solution was placed in the top of the plate and

Siderophore production: - All isolates were assayed for siderophore production on Chrome azurol S agar medium described by (Schwan and Neilands). Chrome azurol S agar medium plates were prepared and spot inoculated with bacterial and actinomycetes isolates and incubated at 28±2^0C for 48 -72 hrs. Development of yellow- orange halo around the growth was considered as positive for Siderophore production.

Antimicrobial activity:- Antifungal and antibacterial activity against plant pathogens of bacterial isolates was studied by agar well diffusion method (Mahalakshmi et al 2009 & Ahmad et al 2008). The bacterial isolates tested for their antifungal activity were fully grown in nutrient broth media. Test fungi were grown on potato dextrose agar. The spores were scraped and suspended in 10ml of sterile normal saline solution. Diluted spore suspension (0.1ml) of the fungi was spread on Muller Hinton

agar. Wells of 10 mm diameter were punched into the agar medium and filled with 200µl of bacterial culture. The plates were incubated at 28 ± 2^0c for 5-6 days for antifungal and 24 hrs for antibacterial activity. The antifungal and antibacterial activity was evaluated by measuring the growth of inhibition zone against test fungi and bacteria.

Agar overlay method: - Actinomycete isolates for the study of antibacterial antifungal activity were spot inoculated on glycerol aspargine agar and plates were incubated for 5-7 days. The isolates were killed by treating them with chloroform in lid for 30 minutes. The plates were overlaid with molten cooled NA and PDA with 0.2 ml of bacterial and fungal cultures respectively. The plates were incubated for 48-72 hrs and observed for zone of inhibition and zone diameters were measured. (Bergey's Manual)

RESULTS :-

In the project work total 41 bacterial and 19 actinomycete isolates were obtained from the rhizosphere of 6 different medicinal plants. Morphological ,cultural biochemical characters and enzymatic potential of isolates was studied. Plant growth promoting activities like NH3, HCN and Siderophore production of all isolates was studied. Antibacterial and antifungal activity against plant pathogens like *Xanthomonas citri, Xanthomonas axenopodis, Fusarium oxysporum and Aspergillus niger* was studied.

Table 1: Morphological characters and enzymatic reactions of bacterial isolates -

Isolate no	**Gram nature**	**Motility**	**Catalase**	**Amylase**	**Caseinase**	**Urease**	**phosphatase**
AM 1	Gram + cocci	Motile	+	+	+	**-ve**	+
AM 2	Gram + rods	Non motile	**-**	+	+	**-**	**-**

AM 3	Gram + rods	motile	+	+	-	+	+
AM 4	Gram + cocci	motile	+	-	-	-	+
AM 5	Gram + rods	Motile	-	+	+	-	+
AM 9	Gram + rods	Non motile	-	+	-	-	+
AM 10	Gram + cocci	Motile	+	-	-	-	-
AMH 2	Gram + rods	Motile	-	+	+	-	+
AMH 4	Gram + rods	Motile	-	+	+	-	-
AMH 7	Gram + rods	Motile	+	+	-	-	-
LA 1	Gram + cocci		+	-	+	+	-
LA 2	Gram + cocci	Motile	+	+	+	-	+

LA 3	Gram + cocci	Non motile	+	-	-	-	+
LA 4	Gram + cocci	Motile	+	-	+	-	+
LA 5	Gram - rods	Motile	-	-	+	-	+
LA 6	Gram + cocci	Motile	+	-	-	-	+
LA 7	Gram + cocci	Motile	+	-	+	-	+
LA 8	Gram + cocci	Motile	+	-	-	-	+
CL 1	Gram + rods	motile	+	+	-	+	-
CL 2	Gram + rods	Nonmotile	+	+	+	+	+
CL 3	Gram + cocci	Motile	+	+	+	+	+
CL 4	Gram + rods	Motile	+	+	+	+	+

CL 5	Gram + rods	Motile	-	+	+	+	+
CL 6	Gram - rods	Motile	+	+	+	+	+
CL 7	Gram+ rods	Motile	+	+	-	+	+
CP 1	Gram + cocci	Motile	+	+	+	+	+
CP 2	Gram + rods	Motile	+	-	+	+	-
CP 3	Gram - coccobacilli	Motile	+	+	+	+	+
CP4	Gram + cocci	Nonmotile	+	-	+	+	-
CP5	Gram + cocci	Motile	+	+	+	+	+
CP 7	Gram + cocci	Motile	+	+	-	+	+
AZ 1	Gram	Nonmoti	+	+	-	-	-

	+ long rods	le					
AZ 2	Gram + rods	Motile	+	+	+	-	-
AZ 3	Gram + rods	Nonmotile	+	-	-	-	-
AZ 4	Gram + cocci	Nonmotile	+	+	+	+	+
AZ 10	Gram + rods	Motile	+	+	+	+	-
AZ 11	Gram + cocci	Motile	+	+	+	-	-
WS 1	Gram + rods	Motile	+	+	+	-	+
WS 2	Gram + rods	Motile	+	+	+	-	-
WS 3	Gram + cocci	Motile	+	+	-	-	-
WS 4	Gram + cocci	Motile	+	-	+	-	-

Table 2: PGPR activities of isolates

Isolate No	NH3	HCN	Sidero phore	Isolate No	NH3	HCN	Sidero phore
AM 1	+	-	-	AMH 2	-	+	+
AM 2	+	+	-	AMH 4	+	+	+
AM 3	+	+	+	AMH 7	+	+	+
AM 4	+	-	-	CP 1	+	+	+
AM 5	+	+	-	CP 2	+	-	+
AM 9	+	+	+	CP 3	+	+	-
AM 10	+	+	+	CP 4	+	+	-
LA 1	+	+	-	CP 5	+	+	+
LA 2	+	+	-	CP 7	+	+	+
LA 3	+	+	-	AZ 1	+	+	+
LA 4	+	+	+	AZ 2	+	-	+
LA 5	+	+	-	AZ 3	+	+	-
LA 6	+	-	+	AZ 4	+	+	-
LA 7	+	+	-	AZ 10	+	+	+
LA 8	+	+	+	AZ 11	+	+	-
CL1	+	+	+	WS 1	+	+	+
CL2	+	+	-	WS 2	+	-	-
CL3	+	-	+	WS 3	+	+	-
CL4	+	-	-	WS 4	+	-	-
CL5	+	-	-				
CL6	+	+	-				
CL7	+	+	-				

Actinomycete isolate No	NH3	HCN	Siderophore	Actinomycete isolate No	NH3	HCN	Siderophore
AM 7	+	+	+	CL 21-2	+	-	-
AMH 2	+	+	+	WS 1	+	+	+
CP 3	+	-	-	WS 2	+	-	-
CP 4	+	-	-	WS 4	+	+	+
CP 5	+	+	+	WS 6	+	-	-
CL 2-2	+	+	+	AZ 8	+	+	+
CL 3-6	+	+	+	AZ 9	+	+	+
CL 3-7	+	-	+	AZ 10A	+	+	+
CL 7-6	+	-	+	AZ 13	+	+	-
CL 8-2	-	+	-				

Table 2:- Antibacterial and antifungal activity of test isolates.

Sr No	**organism**	**Xanthomonas.citri**	**X.axenopodis**	**Fusarium oxysporum**	**Aspergillus niger**
Zone size (mm)					
1	WS 1(bact)	ND	ND	ND	14

2	Streptomyces xanthochromogenes	35	25	12	07
3	Streptomyces pactum	14	19	20	20
4	WS 6 (actino)	20	06	19	12
5	*Streptomyces glaucescens*	19	32	06	16

Plate1,Siderophore production

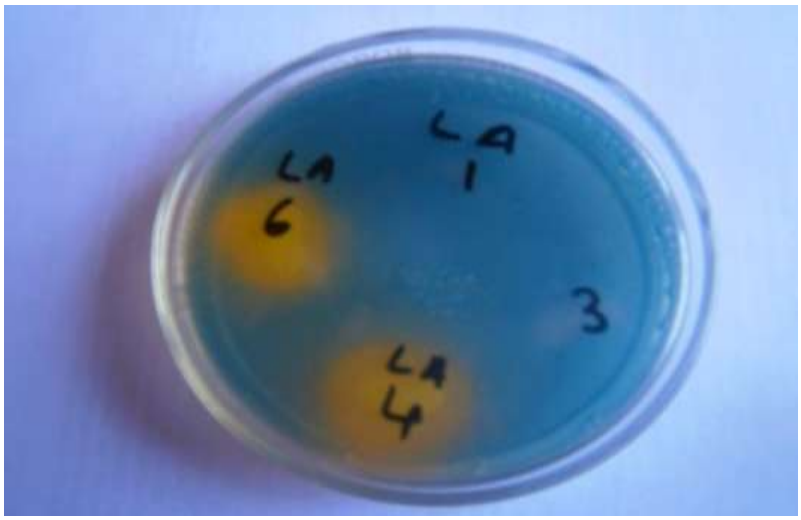
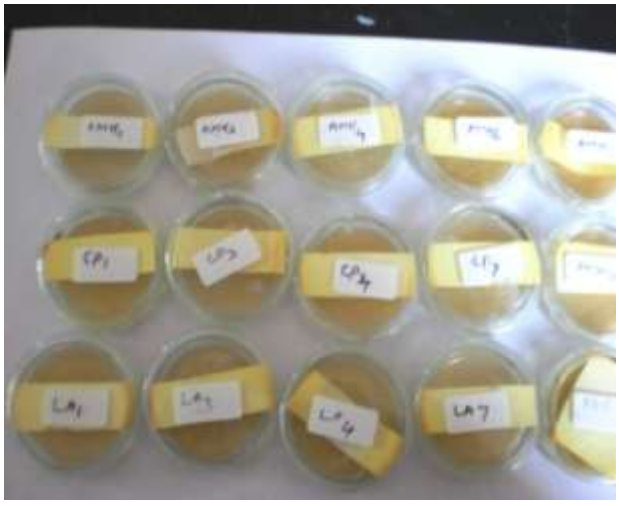


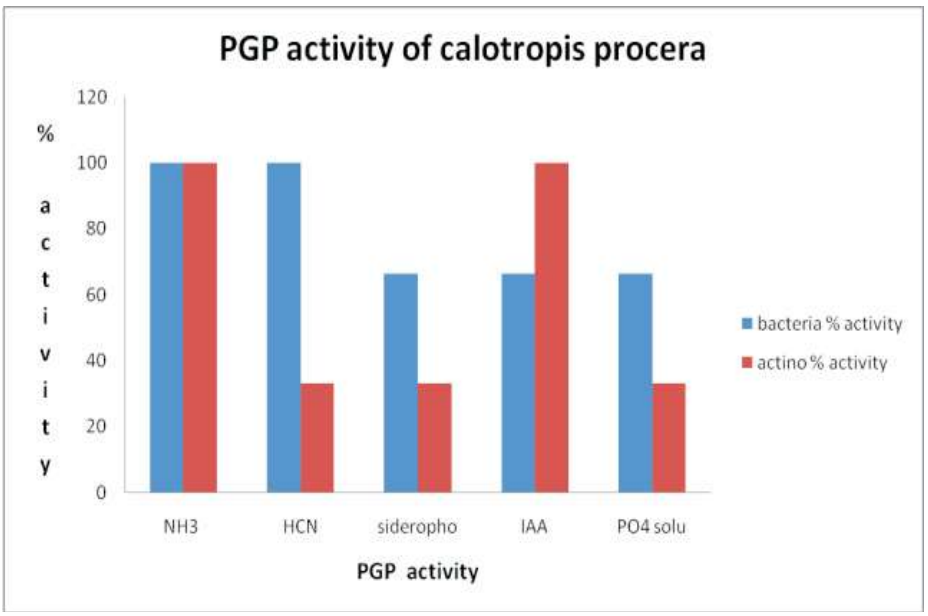
Plate2,Antimicrobial activity of isolates

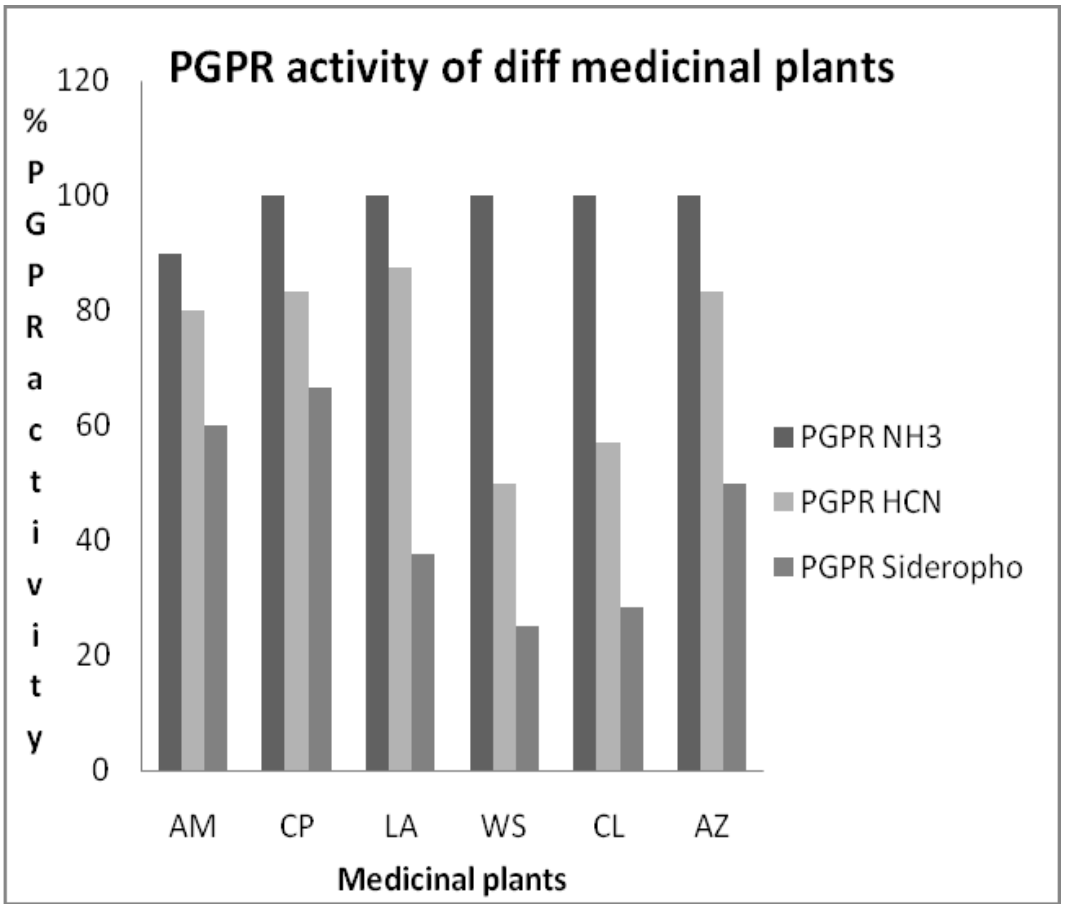
Plate,3 Antifungal activity of bacterial activity

Plate No4 HCN Production



PlateNo.5 NH_3 Production


PGP activity of calotropis procera
% activity
120
100
80
60
40
20
0
NH3
HCN
sideropho
IAA
PO4 solu
PGP activity
bacteria % activity
actino % activity


PGPR activity of diff medicinal plants
% PGPR activity
120
100
80
60
40
20
0
AM
CP
LA
WS
CL
AZ
Medicinal plants
PGPR NH3
PGPR HCN
PGPR Sideropho

Ten bacterial isolates from the rhizosphere of *Aegle marmelos* were studied for NH3, HCN & siderophore production. Out of 10 isolates 5 isolates AM3, AM9, AM10, AMH4 &AMH7 showed all 3 PGP activities, 9 isolates showed NH3 production, 8 isolates HCN production & 6 isolates showed siderophore production. So these isolates showed good PGP activities,

Antimicrobial activity of isolates against plant pathogens showed that isolates AM3 &amh2 significantly inhibited *Xantho. Citri*, *Xanthomonas axenopodis* & *Fusarium oxysporum*. Isolate AM10 showed antimicrobial activity against X.axanopodis, *Fusarium* &*Aspergillus* and no activity against *X.citri*. Isolate AMH1 & AMH7 showed activity against only *Aspergillus* and no other plant pathogens.

8 isolates from the rhizosphere of *Lawsonia alba* were studied for NH3, HCN & siderophore production. Out of 8 isolates 2 isolates LA4 &LA 8 showed all three plant growth promoting activities. Out of 8 isolates, all 8 isolates showed NH3 production, 7 isolates showed HCN production & 3 isolates showed siderophore production.

Results of antimicrobial activity of these isolates were not significant. As only LA3 showed zone of inhibition against Aspergillus niger and LA5 against Fusarium oxysporum.

Total 7 isolates from Curcuma longa were studied for PGP activities.the isolate CL1 showed all 3 PGP activities positive. All 7 isolates produced NH3, 4isolates among seven showed HCN production and 2 isolates showed siderophore production.

Antimicrobial activity of isolates against plant pathogens showed no significant results. Only isolate CL 3 showed antifungal activity against *Aspergillus niger*.

Six isolates from the rhizosphere of *Calotropis procera* were studied for PGP activities. Isolates CP1, CP5 & CP7 showed all three PGP activities positive. All 6 isolates showed NH3 production, 5 isolates produced HCN & 4 isolates showed siderophore production. It is observed from the results that all isolates are good plant growth promoters as well as they have antimicrobial potential. The isolates CP1 & CP3 showed significant inhibitory zones against plant pathogens namely *X. axenopodis*, *Aspergillus niger,* & *Fusarium oxysporum* while isolate CP5 showed inhibitory zone against *X.axenopodis* & *Fusarium oxysporum*.

Six bacterial isolates from *Azadiracta indica* & 4 bacterial isolates from *Withania somnifera* were studied for PGP activities. Among them AZ1, AZ10 & WS1 showed all three i.e NH3, HCN & siderophore production activities positive. All 6 AZ isolates & 4 WS isolates produced NH3,5 AZ and 2 WS isolates produced HCN & 3 AZ and only one WS isolate produced siderophore. Antimicrobial activity against plant pathogens was also studied. The isolate AZ10 showed significant antifungal activity against *A. niger* and *F. oxysporum*, while isolate WS 1 showed antifungal activity against only *Aspergillus*.

Nineteen actinomycetal isolates obtained from five different medicinal plants were also studied for plant growth promoting activities viz. NH3, HCN & siderophore production. Isolates AM7, AMH2, CP5, CL2-2, CL3-6, WS1, WS4, AZ 8, AZ9 &AZ 10A showed all three activities positive. Out of 19 isolates 18 isolates except CL8-2 showed NH3 production positive, 12 isolates showed HCN production and siderophore production positive.

The isolates were also studied for antibacterial and antifungal activity. Isolates obtained from the rhizosphere of *Withania somnifera* plant showed very good antimicrobial activity. Isolates from *Calotropis procera* CP3, CP4 & CP5showed antifungal activity against *Aspergillus niger* & isolates from *Azadiracta indica* also showed antifungal activity against *Aspergillus niger*.

Discussion-

Rhizospheric microflora of plants show various activities like enzymatic potential and plant growth promoting activities like NH_3 ,HCN, IAA and siderophore production. The rhizospheric microorganisms also show antimicrobial activity.

Waseem Safdar et al (2011) studied phosphate solubilizing activity of bacteria and fungi from the rhizosphere of medicinal plants and obtained highest PO_4 solubilizing fungi associated with *Aloe vera* .

Sutthinan Khamna (2010) isolated streptomyces from the rhizosphere of 14 different medicinal plants and found to produce IAA. Malleswari *et al* (2010), isolated bacteria from rhizosphere of different medicinal plants and studied their PGP activities. Mahalakshmi *et al,* (2009) obtained bacterial isolates from rhizosphere of tomato and studied IAA, HCN, NH_3

and siderophore production and po_4 solubilization abilities and also observed that all the bacterial isolates were effective in inhibiting growth of fungal pathogens.

Sutthinan Khamna (2008) and Thangapandian (2007) isolated actinomycetes from the rhizosphere of medicinal plants and reported positive IAA production and identified most of the actinomycetes as Streptomyces.

Mahalakshmi *et al.,* (2009) isolated bacterial isolates from rhizosphere of tomato and studied IAA, HCN, NH_3 and siderophore production and po_4 solubilization abilities and also observed that all the bacterial isolates were effective in inhibiting growth of fungal pathogens.

Tamilarasi et al enumerated diversity of rhizosphere bacteria, actinomycetes &fungi from the rhizosphere & non rhizosphere soil of 50 different medicinal plants and found that in all plants population of microorganisms in rhizosphere soil is higher than non rhizosphere soil , among microorganisms bacterial population was higher in number than fungi and actinomycetes . He also studied enzymatic activities and different PGPR activities of rhizobacteria such as phosphate solubilization IAA production .

The rhizospheric bacterial isolates showed antibiotic resistance to 14 different commercially used antibiotics.

Malleswari D & Bagyanarayana isolated bacterial isolates from different medicinal plants & screened the isolates for PGP activities like IAA, HCN and NH_3 production, PO_4 solubilization ,enzymatic activity and antifungal activity against Macrophomina phaseolina.

In seed germination all 38 isolates showed enhancement of growth promotion in sorghum, green gram & maize.

Malleswari isolated 219 bacterial isolates from the rhizospheric soil of different medicinal plants, studied PGP activities and antifungal activities against *Macrophomina phaseolina*. She also studied enhancement of plant growth by seed inoculation technique and concluded that use of PGPR as inoculants or biofertilizers is an efficient alternative to replace chemical fertilizers & may be used to enhance growth and production of medicinal plants.

Ajay Kumar et al isolated 30 bacterial isolates from the from the French bean rhizospheric soils and screened for in vitro study of different PGPR activities like PO_4 solubilization, IAA ,HCN and NH_3 production ,ACC deaminase &catalase activity.

S Mahalaxmi &D Reetha isolated bacterial isolates like *Azospirillum , Azotobacter Pseudomonas and Bacillus* from the rhizosphere of tomato plant and all the isolates were screened for their PGP activities like IAA ,HCN ,NH_3& siderophore production & PO_4 solubilization ACC deaminase activity and antifungal activity against *R. solani & Fusarium oxysporum* and found many isolates active against these fungi.

Farah Ahmad et al isolated 72 bacterial isolates from the rhizosphere of different crop plants like mustard ,wheat, sugarcane, brinjal, onion, cauliflower ,cabbage and chickpea and screened for in vitro PGP activities like different IAA ,HCN ,NH_3& siderophore production

Our bacterial results are on the same track of the previous observations made by these workers .But our actinomycetal results are unique no one had reported their tested activities earlier hence are maiden reports.

Conclusion - From the present study it can be concluded that rhizosphere of medicinal plants like *Withania somnifera, Aegle marmelos, Calotropis procera* and *Lawsonia alba* harbor bacteria and actinomycetes showing various PGP activities and also antibacterial and antifungal activities against plant pathogens. Hence these organisms can be used as sources of plant growth promoting substances and may be used as biocontrolling agents after field trials.

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Output of the project work

1. **Manpower trained -**

The laboratory assistant and laboratory attendants were trained for media preparation, sterilization and soil sample collection disposal of laboratory wastes during my project work

2. **Registration for Ph.D. degree course-**

I have registered for Ph.D. degree course and I am extending the research work for future studies of the same topic which I have chosen for present project work. I am enclosing herewith the copy of my admission letter for Ph. D course issued by Solapur University Solapur &copy of first page of synopsis for Ph. D course.

Research publication

Title of the research article	Name of the journal	
Enzymatic potential of bacteria isolated from the rhizosphere of Aegle marmelos (Bael tree)	World journal of Environmental Biosciences vol.1,issue 2	Published
Screening of rhizomicroflora from the rhizosphere of *Calotropis procera* for their multiple plant growth promoting and antimicrobial activity.	Indian Streams Research Journal Vol 3 Issue 10 Nov 2013	Published

Principal investigator - Mrs Nilima Raghunath Damle

UGC approval no - 47/1624/10 (WRO) Pune 1st May 2011

Title of the Project - Studies on potential of rhizobacteria associated with medicinal plant roots for biocontrol of common crop diseases.

Executive summary

The present study was intended to isolate plant growth promoting microorganisms from the rhizosphere of 6 different medicinal plants viz. *Aegle marmelos, Calotropis procera, Azadirachta indica, Withania somnifera, Lawsonia alba* and *Curcuma longa* . Total 41 bacterial and 19 actinomycetal isolates were obtained from the rhizosphere of 6 medicinal plants. All the isolates were characterized on the basis of morphological, cultural and biochemical characters. The isolates having biocontrolling potential were also identified by 16 S rRNA All the isolates were screened for plant growth promoting activities namely NH_3, HCN and siderophore production and also tested in vitro for antimicrobial activity against plant pathogens. The results showed that 100 % bacterial isolates except 1 isolate from *Aegle marmelos* produced NH3, more than 50% isolates produced HCN and siderophore production was shown by 66.6 % isolates from *Aegle marmelos* and *Calotropis procera*. 25-50 % isolates from remaining plants showed siderophore production.

Results of antimicrobial activity against plant pathogens showed significant results with the isolates obtained from *Calotropis procera* and *Aegle marmelos.*

Among 19 actinomycetal isolates 94.21% isolates produced NH_3, 63.1% isolates produced HCN and siderophore.

The results of PGP activities of isolates showed that many bacterial and actinomycetal isolates produced more than one PGP substance. Thus these isolates can be used as bioinoculants as biofertilizers as they have produced many plant growth promoting substances and plant growth can be improved.

Results of antimicrobial activity against plant pathogens showed significant results with the isolates obtained from *Withania somnifera and Calotropis procera* and biocontrolling agents and hence are agriculturally important. Some of the isolates can be used as bio controlling agents to control diseases like Oily spots of pome granate, citrus canker and diseases caused by fungi *Fusarium oxysporum and Aspergillus niger*.