# DIVERSITY STATUS OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) IN RHIZOSPHERE OF SELECTED MEDICINAL PLANTS IN KANYAKUMARI DISTRICT, TAMIL NADU

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## *ABSTRACT*

*In the present study, an attempt was made to investigate the diversity status of different beneficial microbes such as Arbuscular Mycorrhizal (AM) fungi and Plant Growth Promoting Rhizobacteria (PGPR) associated with 36 different medicinal plants growing in Kanyakumari district, Tamil Nadu. An attempt was made to determine the diversity status of Plant Growth Promoting Rhizobacteria in association with the rhizosphere of 36 different medicinal plants, for selection of potential bio-inoculants based on their growth hormone production and phosphate solubilization efficiency for application in nursery and field. All the PGPR isolates isolated from the rhizosphere of 36 different medicinal plants were screened for the IAA production and phosphate solubilisation efficacy under in vitro condition and some of the isolates revealed excellent performance. Those isolates were pure cultured and maintained for further screening their bio-control ability and nursery application to different medicinal plants for quality plants production.*

***KEYWORDS:*** *Arbuscular Mycorrhizal, efficiency, medicinal plants, rhizosphere and solubilisation.*

## Introduction

Medicinal plants are the oldest form of healthcare known to mankind and from the ancient time people are using different herbs or plants as the remedy for various diseases. Medicinal plants are very good resources of new drugs. Many food crops have medicinal effects and modern medicines are produced indirectly from medicinal plants. Medicinal plants are also used for its antibacterial, antifungal and antiviral activities. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine. But, urban people have become dependent on synthetic medicines which have many side effects. So to reduce the side effects we can use medicinal plants for the treatment of common diseases rather than using synthetic drugs. India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani and Siddha traditional medicines.

Microorganisms are ubiquitous in nature and form vital components of all known ecosystems on earth. Their ubiquity is attributed mainly to the small size, easy dispersal, ability to survive and multiply in diverse habitats, including anaerobic and other extreme conditions, their metabolic versatility and flexibility to utilize wide substrates as nutrient source. Actinomycetes, Bacteria and Fungi are the three major groups of soil inhabiting microorganisms. Diverse vegetation, including medicinal herbs, shrubs and trees harbour selected groups of soil microorganisms. Bio-fertilizers or microbial inoculants can be defined as biologically active products containing active strains of selective micro organisms like bacteria, fungi and algae alone or in combination, which may help in increasing plant productivity by way of helping in the biological nitrogen fixation, solubilisation of the insoluble chemical fertilizer material, stimulating plant growth or in decomposition of plant residues. These bio-inoculants or bio-fertilizers are beneficial microorganisms such as Plant Growth Promoting Rhizobacteria (PGPR), Arbuscular Mycorrhizal (AM) fungi, Ectomycorrhizal (E M) fungi, *Frankia, Rhizobium* etc., which are involved in breakdown of organic matter, $N_2$ fixation and secretion of plant growth substances and increase of available mineral nutrients in soil.

Among different beneficial microbes, Plant Growth Promoting Rhizobacteria (PGPR) is a group of beneficial bacteria that actively colonize plant roots and enhance plant growth and biomass. The mechanisms by which PGPR promote the plant growth, include the ability to produce plant growth hormones, asymbiotic N fixation - against plant pathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal synthesis of antibiotics, enzymes and/or fungicidal phosphates and other nutrients (Yang *et al.,* 2009). According to Bashan (1998) intensive and extensive interactions have been established between soil microorganisms and various other soil organisms, including plant roots and plant growth promotion by rhizosphere microorganisms is well established.

Since no much report is available on the diversity of PGPRs in the rhizosphere of different medicinal plants, the present study was undertaken to ascertain the biodiversity status of the PGPR in association with thirty six (36) different medicinal plants and also determine the population density of these organisms.

**Methodology**

**Medicinal plants species selected under study**

In the present study, thirty six medicinally important plant species of herbs, shrubs and few tree species belonging to twenty four families were selected (Table 1). Among them, the families Acanthaceae, Asteraceae, Lamiaceae and Solanaceae have 3 plant species each and the families Rutaceae, Verbenaceae, Asclepiadaceae and Combretaceae having 2 plant species each and the remaining 16 families having 1 plant species.

**Table 1. List of medicinal plants selected for the study**

| S.No. | Name of the Medicinal Plants | Common Name | Family |
|---|---|---|---|
| 1 | *Acorus calamus* | Vasambu | Acoraceae |
| 2 | *Adathoda vasica* | Adathodai | Acanthaceae |
| 3 | *Aegle marmelos* | Vilvam | Rutaceae |
| 4 | *Aristolochia bracteolata* | Aduthinnarppalai | Aristolochiaceae |
| 5 | *Azadirachta indica* | Neem, Vembu | Meliaceae |
| 6 | *Centella asiatica* | Vallarai | Apiaceae |
| 7 | *Cichorium intibus* | Kasinikkerai | Asteraceae |
| 8 | *Eclipta prostrata* | Karisilanganni | Asteraceae |
| 9 | *Gmelina arborea* | Kumil Thekku | Verbenaceae |
| 10 | *Gymnema sylvestre* | Chakkarakolli | Asclepiadaceae |
| 11 | *Hemidesmus indicus* | Nannaari | Asclepiadaceae |
| 12 | *Hibiscua rosa-sinensis* | Chembaruthi | Malvaceae |
| 13 | *Hygrophylla auriculata* | Nir-mulli | Acanthaceae |
| 14 | *Kalanchoe pinnata* | Air plant, Runakkalli | Crassulaceae |
| 15 | *Limonia elephantum* | Wood Apple, Vilanga | Rutaceae |
| 16 | *Ocimum basilicum* | Tulasi; Tirunittru | Lamiaceae |
| 17 | *Ocimum sanctum* | Thulasi; Holy Basil | Lamiaceae |
| 18 | *Oxalis corniculata* | Puli-yarai | Oxalidaceae |
| 19 | *Phyllanthus emblica* | Nelli; Indian Gooseberry | Phyllanthaceae |
| 20 | *Phyla nodiflora* | Podutalei | Verbenaceae |
| 21 | *Plectranthus amboinicus* | Karpuravalli | Lamiaceae |
| 22 | *Rauvolfia tetraphylla* | Pampukaalaachchedi | Apocyanaceae |
| 23 | *Solanum trilobatum* | Tuduvalai | Solanaceae |
| 24 | *Santalum album* | Sandal wood; | Santalaceae |
| 25 | *Strychnos nox-vomica* | Etti Maram | Loganiaceae |
| 26 | *Tinospora cornifolia* | Kunali | Menispermaceae |
| 27 | *Withania somnifera* | Ashwagandha; Amukkuram | Solanaceae |
| 28 | *Aloe vera* | Kathalai | Asphodelaceae |
| 29 | *Andrographis paniculata* | Nilavembu | Acanthaceae |
| 30 | *Curcuma longa* | Manjal; Turmeric | Zingiberaceae |
| 31 | *Tridax procumbens* | Vettukkaya thalai | Asteraceae |
| 32 | *Chrysopogon zizanioides* | Vetiver | Poaceae |
| 33 | *Costus igneus* | Insulin plant; Neyccarikamaram | Costaceae |
| 34 | *Syzygium cumini* | Naval Maram; Jamun | Myrtaceae |
| 35 | *Terminalia bellerica* | Thandri Maram | Combretaceae |
| 36 | *Terminalia chebula* | Kadukkai Maram | Combretaceae |

**Collection of Samples**

Roots and rhizosphere soil samples were collected under the root zone of all the 36 different medicinal plants of the selected study site in Kanyakumari district, Tamil Nadu in clean zip lock polythene covers, sealed tightly and immediately transported to Botany Laboratory, S.T. Hindu College, Nagercoil. The samples were kept in refrigerator at 4ºC until further use.

**Estimation of physico-chemical parameters of soil samples**

Soil samples were analyzed for their physico-chemical parameters such as pH, Electrical Conductivity (EC), available Nitrogen (N), Phosphorus (P), Pottassium (K) and micronutrients such as Copper (Cu), Zinc (Zn), Magnesium (Mg) and Manganese (Mn) following standard laboratory methods.

**Enumeration of total rhizosphere micro flora**

Rhizosphere micro flora were isolated and estimated from the samples of selected medicinal plants by spread plate technique. Jensen's medium, Pikovskaya's agar medium, Rojo Congo medium for bacteria, starch casein agar media for Actinomycetes and potato dextrose agar for fungi were used and the plates were incubated at 37ºC. Colony forming units (CFU/g) were calculated after 24 h for bacteria, and 72 to 96 h for fungi and actinomycetes. Morphologically different colonies were picked and streaked on to the respective medium to obtain pure culture and they were stored at -20ºC for further analysis (Subba Rao, 1995).

**Isolation of Actinomycetes from collected samples**

Serial dilution of soil samples was made using sterile distilled water. The soil suspensions were plated using Starch Casein Agar (SCA) medium supplemented with 100µg/ml cycloheximide. Then the plates incubated at 30ºC for 7-10 days. Emerging Actinomycetes were picked and streaked onto fresh SCA plates and incubated at 30ºC for 7 days. Pure cultures of the Actinomycetes isolates were maintained in SCA slants and stored at refrigerator.

**Isolation of PGPRs from rhizosphere soil sample**

Serial dilution and plating techniques as described by Parkinson *et al*. (1971) and Subba Rao (1993) were adopted for enumerating the status of PGPR. Among different PGPRs, *Azotobacter* colonies were selected based on the appearance of mucoid, transparent, gummy colonies; *Azospirillum* colonies appeared as scarlet pink, round colonies and phosphate solubilising bacteria (PSB) were identified based on the halo zone formed around the colonies. Population density of PGPRs was also determined for each sample as CFU/g (Colony Forming Units/gm of soil) (Rodriguez, 1982; Subba Rao, 1993) and statistically analyzed using ANOVA by SPSS10.0 version. All the isolates of PGPR *viz.*, *Azotobacter*, *Azospirillum* and phosphate solubilising bacteria were maintained in nutrient agar slants at 4ºC for further studies.

**Maintenance of PGPR cultures**

All the isolates of PGPR *viz.*, *Azotobacter*, *Azospirillum* and Phosphate Solubilizing Bacteria were maintained in nutrient agar slants at 4ºC (Cappuccino and Sherman, 1996) for further studies.

**Determination of Indole Acetic Acid (IAA) Production by PGPR**

An experiment was conducted to determine plant growth hormone production by different PGPR isolates by adapting the method described by (Bent *et al.,* 2001). The tubes containing nutrient broth with tryptophan (2mg/ml) was sterilised and inoculated with selected bacteria, after incubation, the culture broth was taken by 0.5ml and adding 1.5ml of sterile distilled water. To this 4 ml of freshly prepared Salkowasky's reagent (50 ml of 35% per chloric acid + 1ml of 0.5M Ferric chloric acid) was added. The tubes were incubated in dark for 30 minutes for the development of pink coloured complex. After 30 minutes, the absorbance was measured at 530nm. IAA concentration in the culture was determined by using a calibration curve of pure IAA as a standard.

**Determination of Phosphate solubilization by PSBs**

The microbes isolated from medicinal plant soil samples were retested by plate assay for phosphate solubilization in Pikovskaya's agar medium. The bacteria were sporting on the medium. The halo zone around the colony was presumptive conformation of phosphate solubilization and was measured after 7 days of incubation at 30º ± 1º. Halo size was calculated by subtracting colony diameter from the total halo zone. Solubilisation efficiency (SE) was calculated by the following formula as described by Sharma *et al.* (2007):

$$\text{Solubilisation Efficiency (SE)} = \frac{\text{Growth diameter}}{\text{Solubilisation diameter}} \times 100$$

**Results and Discussion**

The rhizosphere is a dynamic soil environment formed by living plant roots and their associated microflora. The beneficial microbes are utilized as bio-fertilizers or bio-inoculants. They not only improve plant growth but also maintain the soil fertility. Since these bio-resources represent a great diversity in chemical, physical and biological characteristics, their efficient use depend on, among others, identification of suitable type of bio-fertilizers or bio-inoculants. Towards these goals, an attempt was made to determine the diversity status of Plant Growth Promoting Rhizobacteria in association with the rhizosphere of 36 different medicinal plants, for selection of potential bio-inoculants based on their growth hormone production and phosphate solubilization efficiency for application in nursery and field.

Data on physico-chemical properties of soil samples collected from different medicinal plants in selected study locations at Kanyakumari is presented in Table 2. It was observed that the study location displayed slightly acidic to neutral pH. The electrical conductivity which represents total ion concentration was ranged of 0.23-0.41. The bulk density was found as 1.12–1.36 gm/cc. The available nitrogen was observed low to moderate and available phosphorus content was less as compared to potassium. In the present study, calcium and magnesium level was very low. Soil texture is found as sandy to sandy loam.

**Table 2. Physico-chemical properties of rhizosphere soil samples of different medicinal plants collected from Kanyakumari District, Tamil Nadu**

| S.No. | Parameters | Soil samples collected under different medicinal plants |
|---|---|---|
| 1 | pH | 5.01 to 6.63 |
| 2 | EC (ds/m) | 0.23 - 0.41 |
| 3 | Bulk Density (gm/cc) | 1.12 – 1.36 |
| 4 | Organic Carbon (%) | 0.77 - 2.13 |
| 5 | Available Nitrogen (kg/ha) | 84.6 - 176.4 |
| 6 | Available Phosphorus (kg/ha) | 19.9 - 46.7 |
| 7 | Available potassium (kg/ha) | 177.9 - 265.8 |
| 8 | Calcium (Meq/100g) | 4.9 – 9.6 |
| 9 | Magnesium (Meq/100g) | 5.3 - 6.5 |
| 10 | DTPA-Cu (ppm) | 0.30 - 0.63 |
| 11 | DTPA-Zn (ppm) | 0.45 – 1.02 |
| 12 | DTPA-Mn (ppm) | 0.53 – 1.53 |
| 13 | DTPA-Fe (ppm) | 0.75 - 2.21 |
| 14 | Texture | Sandy to Sandy loam |

Similar kind of results was reported in tropical soils characterized by low nutrient status according to Kang and Wilson (1987). Similarly, Ritsema and Dekker (1994) and Dekker and Ritsema (1996) have observed the variation in soil nutrient availability with space and time.

**Enumeration of Rhizosphere Microorganisms**

The population density of Plant Growth Promoting Bacteria (PGPR), Fungi and Actinomycetes was counted and calculated using colony forming units (CFU) **(Tables 3a and 3b).** A total of 1,718 colonies of different microbes were obtained from the rhizosphere soil samples of 36 different medicinal plants during the period of investigation. Of which, 1,229 colonies were PGPR organisms (*Azotobacter*, *Azospirillum* and Phosphobacteria), 270 colonies were Actinomycetes and 219 colonies of soil Fungi. The population densities of rhizobacterial (PGPR) colonies were observed to be high. Among PGPR colonies, maximum PGPR population was recorded from the rhizosphere of *Ocimum sanctum* (62 x $10^4$ cfu/g soil), which is followed by *Santalum* album (57 x $10^4$ cfu/g soil), *Kalanchoe pinnata* (54 x $10^4$ cfu/g soil) and *Gmelina arborea* (52 x $10^4$ cfu/g soil), Less population of different PGPR colonies was recorded from the rhizosphere of *Gymnema sylvestre* (6 x $10^4$ cfu/g soil), *Cichorium intibus* and *Tinospora cornifolia* (7 x $10^4$ cfu/g soil) each and *Strictus nux-vomica* (8 x $10^4$ cfu/g soil). Population of *Actinomycetes* colonies was found more from *Cemtella asiatica* (18 x $10^4$ cfu/g soil), followed by *Ocimum sanctium* (17 x $10^4$ cfu/g soil), *Acorus calamus* and *Plectranthus amboinicus* (16 X $10^4$ cfu/g soil) each and *Gmelina arborea* (15 x $10^4$ cfu/g soil). Less population of Actinomycetes was recorded from the rhizosphere of *Oxalis corniculta* (2 x $10^4$ cfu/g soil), followed by *Aegle marmelos, Limonia elephantum, Tinospora cornifolia, Costeus igneus* and *Terminalia bellerica* (3 x $10^4$ cfu/g soil) each. No

Actiomycete population was recorded from the rhizosphere samples of *Aristolochia bracteata*, *Hygrophylla auriculata* and *Phyllanthus emblica* during the period of study. There is a variation in population density of soil fungi from the rhizosphere of 36 different medicinal plants screened.

**Table 3a. Population density of different soil microbes from the rhizosphere soil samples of different medicinal plants**

| S.No. | Medicinal Plants | PGPR (CFU/g of soil)* | Actinomycetes (CFU/g of soil)* | Fungi (CFU/g of soil)* |
|---|---|---|---|---|
| 1 | *Acorus calamus* | 47c | 16c | 14b |
| 2 | *Adathoda vasica* | 39c | 9a | 6a |
| 3 | *Aegle marmelos* | 12a | 3a | 8a |
| 4 | *Aristolochia bracteata* | 23b | --- | 7a |
| 5 | *Azadirachta indica* | 30b | 12b | 10b |
| 6 | *Centella asiatica* | 44c | 18c | 5a |
| 7 | *Cichorium intibus* | 7a | 6a | 7a |
| 8 | *Eclipta prostrata* | 30b | 9a | 3a |
| 9 | *Gmelina arborea* | 52d | 15c | 11b |
| 10 | *Gymnema sylvestre* | 6a | 7a | 4a |
| 11 | *Hemidesmus indicus* | 38b | 8a | 2a |
| 12 | *Hibiscua rosa-sinensis* | 49c | 14b | 3a |
| 13 | *Hygrophylla auriculata* | 19a | --- | 9a |
| 14 | *Kalanchoe pinnata* | 54c | 7a | 5a |
| 15 | *Limonia elephantum* | 33b | 3a | 4a |
| 16 | *Ocimum basilicum* | 44c | 6a | 2a |
| 17 | *Ocimum sanctum* | 62d | 17c | 8a |
| 18 | *Oxalis corniculata* | 26c | 2a | 6a |
| 19 | *Phyllanthus emblica* | 17a | --- | 5a |
| 20 | *Phyla nodiflora* | 29b | 6a | 3a |
| | Total | 661 | 158 | 122 |

*Means, in a column, followed by common letter (s) are not significantly different at 0.05 level according to DMRT.

**Table 3b. Population density of different soil microbes from the rhizosphere soil Samples of different medicinal plants**

| S.No. | Medicinal Plants | PGPR (CFU/g of soil)* | Actinomycetes (CFU/g of soil)* | Fungi (CFU/g of soil)* |
|---|---|---|---|---|
| 21 | *Plectranthus amboinicus* | 49d | 16c | 5a |
| 22 | *Rauvolfia tetraphylla* | 28b | 5a | 3a |
| 23 | *Solanum trilobatum* | 30b | 4a | 9b |

| | | | | |
|---|---|---|---|---|
| 24 | *Santalum album* | 57d | 10b | 6a |
| 25 | *Strychnos nox-vomica* | 8a | 7a | 10b |
| 26 | *Tinospora cornifolia* | 7a | 3a | 2a |
| 27 | *Withania somnifera* | 46d | 5a | 11b |
| 28 | *Aloe vera* | 41d | 10b | 3a |
| 29 | *Andrographis paniculata* | 47d | 6a | 5a |
| 30 | *Curcuma longa* | 29b | 11b | 7a |
| 31 | *Solanum procumbens* | 37c | 8a | 6a |
| 32 | *Chrysopogon zizanioides* | 27b | 5a | 9b |
| 33 | *Costus igneus* | 21b | 3a | 6a |
| 34 | *Syzygium cumini* | 43d | 10b | 4a |
| 35 | *Terminalia bellerica* | 50d | 3a | 2a |
| 36 | *Terminalia chebula* | 48d | 6a | 9b |
| Total | | 568 | 112 | 97 |
| Total population density of 20 (twenty) different medicinal plants (as per Table 3a) | | 661 | 158 | 122 |
| **Grand Total (Total population density of all 36 different medicinal plants)** | | 1229 | 270 | 219 |

*Means, in a column, followed by common letter (s) are not significantly different at 0.05 level according to DMRT

**Population density status of Plant Growth Promoting Rhizobacteria from the rhizosphere of different medicinal plants**

The population density of different Plant Growth Promoting Bacteria (PGPR) organisms such as *Azotobacter, Azospirillum* and Phosphobacteria from the rhizosphere of 36 different medicinal plants was estimated and the data is presented in **Tables 4a and 4b.**

**Table 4a. Population density status of Plant Growth Promoting Rhizobacteria (PGPR) from the rhizosphere soil samples of different medicinal plants**

| S. No. | Medicinal Plant Name | Population density of PGPR organisms* | | | Total colonies (CFU/g) |
|---|---|---|---|---|---|
| | | *Azotobacter* | *Azospirillum* | Phosphobacterium | |
| 1 | *Acorus calamus* | 12b | 18c | 17c | 47 |
| 2 | *Adathoda vasica* | 5a | 19c | 15c | 39 |
| 3 | *Aegle marmelos* | 2a | 5a | 5a | 12 |
| 4 | *Aristolochia bracteata* | 3a | 9a | 11b | 23 |
| 5 | *Azadirachta indica* | 4a | 10b | 16c | 30 |
| 6 | *Centella asiatica* | 7a | 18c | 19c | 44 |
| 7 | *Cichorium intibus* | 2a | 2a | 3a | 7 |
| 8 | *Eclipta prostrata* | 6a | 10b | 14b | 30 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | *Gmelina arborea* | 12b | 18c | 22d | 52 |
| 10 | *Gymnema sylvestre* | 1 | 3a | 2a | 6 |
| 11 | *Hemidesmus indicus* | 10b | 16c | 12b | 38 |
| 12 | *Hibiscua rosa-sinensis* | 18c | 12b | 19c | 49 |
| 13 | *Hygrophylla auriculata* | 4a | 8 | 7 | 19 |
| 14 | *Kalanchoe pinnata* | 12b | 19c | 23d | 54 |
| 15 | *Limonia elephantum* | 8a | 11b | 14b | 33 |
| 16 | *Ocimum basilicum* | 15c | 19c | 10b | 44 |
| 17 | *Ocimum sanctum* | 19c | 18c | 25d | 62 |
| 18 | *Oxalis corniculata* | 7a | 10b | 9a | 26 |
| 19 | *Phyllanthus emblica* | 4a | 7a | 6a | 17 |
| 20 | *Phyla nodiflora* | 9a | 12b | 8a | 29 |
| | Total | 160 | 244 | 257 | 661 |

*Means, in a column, followed by common letter (s) are not significantly different at 0.05 level according to DMRT

**Table 4b. Population density status of Plant Growth Promoting Rhizobacteria (PGPR) from the rhizosphere soil samples of different medicinal plants**

| S. No. | Medicinal Plant Name | Population of PGPR organisms* | | | Total colonies (CFU/g) |
|---|---|---|---|---|---|
| | | ***Azotobacter*** | ***Azospirillum*** | **Phosphobacterium** | |
| 21 | *Plectranthus amboinicus* | 9a | 18c | 22d | 49 |
| 22 | *Rauvolfia tetraphylla* | 9a | 10b | 9a | 28 |
| 23 | *Solanum trilobatum* | 7a | 9a | 14b | 30 |
| 24 | *Santalum album* | 11b | 19c | 27d | 57 |
| 25 | *Strychnos nox-vomica* | 3a | 3a | 2a | 8 |
| 26 | *Tinospora cornifolia* | 2a | 2a | 3a | 7 |
| 27 | *Withania somnifera* | 9a | 15c | 22d | 46 |
| 28 | *Aloe vera* | 8a | 14b | 19c | 41 |
| 29 | *Andrographis paniculata* | 7a | 18c | 22d | 47 |
| 30 | *Curcuma longa* | 4a | 13b | 12b | 29 |
| 31 | *Solanum procumbens* | 11b | 14b | 12b | 37 |
| 32 | *Chrysopogon zizanioides* | 4a | 10b | 13b | 27 |
| 33 | *Costus igneus* | 5a | 7a | 9a | 21 |
| 34 | *Syzygium cumini* | 9a | 18c | 16c | 43 |
| 35 | *Terminalia bellerica* | 11b | 18c | 21d | 50 |
| 36 | *Terminalia chebula* | 12b | 16c | 20d | 48 |
| | Total | 121 | 204 | 243 | 568 |
| Total population density of 20 different medicinal plants (as per Table 4) | | 160 | 244 | 257 | 661 |
| | **Grand Total** | **281** | **448** | **500** | **1229** |

| | | | | |
|---|---|---|---|---|
| **(Total population density of 36 (thirty six) different medicinal plants)** | | | | |

*Means, in a column, followed by common letter (s) are not significantly different at 0.05 level according to DMRT

It was observed that population density of Phosphobacteria was found maximum (500 x $10^4$ cfu/g soil) and this is followed by *Azospirillum* colonies (448 x $10^4$ cfu/g soil) and *Azotobacter* colonies (281 x $10^4$ cfu/g soil). It was also found that maximum Phosphobacteria colonies was recorded from the rhizosphere of *Santalum album* (27 x $10^4$ cfu/g soil), followed by *Ocimum sanctium* (25 x $10^4$ cfu/g soil), *Kalanchoe pinnata* (23 x $10^4$ cfu/g soil) and *Gmelina arborea, Plectranthus amboinicus, Withania somnifera* and *Andrographis paniculata* (22 x $10^4$ cfu/g soil) each. Less population of Phosphobacteria was recorded from the rhizsphere of Strictus nux-vomica (2 x $10^4$ cfu/g soil), followed by Tinospora cornifolia (3 x $10^4$ cfu/g soil).

Maximum *Azospirillum* colonies was recorded from the rhizosphere of *Adathoda vasica, Kalanchoe pinnata, Ocimum basilicum* and *Santalum album* (19 x $10^4$ cfu/g soil) each, followed by *Acorus calamus, Centella asiatica, Gmelina arborea, Ocimum sanctum, Plectranthus amboinicus, Andrographis paniculata, Syzygium cumini* and *Terminalia bellerica* (18 x $10^4$ cfu/g soil) each respectively. Less population of *Azospirillum* was recorded from the rhizosphere of *Tinospora cornifolia* (2 x $10^4$ cfu/g soil), followed by *Strictus nux-vomica* (3 x $10^4$ cfu/g soil) during the period of study.

Maximum *Azotobacter* colonies was recorded from the rhizosphere of *Ocimum sanctum* (19 x $10^4$ cfu/g soil), followed by *Hibiscus rosa-sinensis* (18 x $10^4$ cfu/g soil) and *Gmelina arborea* (17 x $10^4$ cfu/g soil). Less *Azotobacter* colonies was recorded from the rhizosphere of *Gymnema sylvestre* (1 x $10^4$ cfu/g soil), followed by *Aegle marmelos, Cichorium intibus* and *Tinospora cornifolia* (2 x $10^4$ cfu/g soil) each and *Strictus nux-vomica* and *Aristolochia bracteata* (3 x $10^4$ cfu/g soil) during the study period.

***In vitro* screening of different isolates of PGPR for Indole acetic acid production**

An experiment was conducted to determine the efficacy of plant growth hormone production by selected 4 different isolates of PGPR under *in vitro* and data is presented in **Table 5.** It was found that all the selected isolates are able to produce IAA production but there is a variation in quantity of production by different isolates. It was recorded that the isolate Nos. 27 and 45 showed maximum amount of IAA production as compared to other isolates.

**Table 5. Screening of different isolates of PGPR for Indole Acetic Acid production**

| S.No. | Isolation code | IAA production (µg/ml) |
|---|---|---|
| 1 | 1 | 0.00922 |
| 2 | 6 | 0.01055 |
| 3 | 9 | 0.00657 |
| 4 | 10 | 0.01123 |
| 5 | 15 | 0.00212 |

| | | |
|---|---|---|
| 6 | 27 | 0.03791 |
| 7 | 45 | 0.09292 |
| 8 | 55 | 0.00113 |
| 9 | 57 | 0.02112 |
| 10 | 60 | 0.00201 |

***In vitro* screening of different isolates of PGPR for phosphate solubilisation**

All the selected phosphobacterial isolates were screened for their phosphate solubilisation efficiency using Pikovskaya's agar medium supplemented with calcium phosphate in laboratory. The colony and zone size of different isolates is shown in **Table 6.** It was observed that all the isolates revealed high "P" solubilisation efficiency. Maximum phosphate solubilisation efficiency was observed for isolate Nos. 27, 45 of different samples screened.

**Table 6. Phosphate Solubilisation efficiency from PGPR isolates**

| S. No. | Isolation code | Colony size (mm) | Zone size (mm) | Result (mm) |
|---|---|---|---|---|
| 1 | 10 | 17 | Nil | Nil |
| 2 | 11 | 14 | Nil | Nil |
| 3 | 12 | 18 | Nil | Nil |
| 4 | 14 | 13 | Nil | Nil |
| 5 | 15 | 15 | Nil | Nil |
| 6 | 16 | 11 | Nil | Nil |
| 7 | 18 | 19 | Nil | Nil |
| 8 | 19 | 13 | Nil | Nil |
| 9 | 20 | 15 | Nil | Nil |
| 10 | 22 | 13 | Nil | Nil |
| 11 | 27 | 6 | 35 | 83.3 |
| 12 | 45 | 3 | 17 | 66.6 |
| 13 | 46 | 18 | Nil | Nil |
| 14 | 50 | 16 | Nil | Nil |
| 15 | 52 | 11 | Nil | Nil |
| 16 | 53 | 14 | Nil | Nil |
| 17 | 55 | 18 | Nil | Nil |
| 18 | 57 | 12 | Nil | Nil |
| 19 | 60 | 19 | Nil | Nil |
| 20 | 63 | 13 | Nil | Nil |

The findings of the study are corroborated with the findings made by Joseph *et al.* (2007) and Farah *et al.* (2008). Their study also showed the potential of such PGPR isolates in their plant growth promoting traits like production of Indole Acetic Acid (IAA), Ammonia (NH3), Hydrogen Cyanide (HCN), siderophore and catalase.

## CONCLUSION

All the PGPR isolates isolated from the rhizosphere of 36 different medicinal plants were screened for the IAA production and phosphate solubilisation efficacy under *in vitro* condition and some of the isolates revealed excellent performance. Those isolates were pure cultured and maintained for further screening their bio-control ability and nursery application to different medicinal plants for quality plants production.

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