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# Effect on the plant growth promoting rhizobacteria (PGPR) increasing plant height, chlorophyll and protein content of *Solanum nigrum*

**Sivaprakasam Megala and Paranthaman**

**Abstract**
The effect of microbial consortium consisting plant growth promoting rhizobacteria (PGPR) like *Azospirillum lipoferum, Azotobacter chroococcum, Bacillus megaterium, Pseudomonas fluorescens* were tested separately and in combination of *Solanum nigrum.* The combinations of above mentioned PGPR strains significantly increased plant height, chlorophyll and protein content in *Solanum nigrum*, when compared to the uninocultated control. The microbial consortium ($T_{11}$) increased in plant height, chlorophyll and protein content. Which recorded consortium ($T_{11}$) 166.00 cm for plant height on atharvest, 5.96 mg $g^{-1}$ for chlorophyll and 13.46 mg $g^{-1}$ for protein content on 120 DAS.

**Keywords:** *Solanum nigrum*, PGPR, plant height, chlorophyll, protein content

## 1. Introduction
Plant derived medicines have been part of traditional health care in most parts of the world for thousands for year (Palombo and Semple, 2001) [15]. More than 80 % of the population mainly in developing countries depends on plants for their medicinal needs (Farmsworth, 1988). In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous medicinal plants and their therapeutic values (Jhon Britto *et al*., 2002) [10]. Medicinal plants occupies a special place in economic production, especially at the present time where it is considered one of the most important strategic materials in the pharmaceutical industry or rather nucleus of the prefix in the chemical composition of the drug (Djaafar and Ridha, 2014) [5].

*Solanum nigrum* commonly known as "Black nightshade" belongs to Solanaceae family. It is known as Manathakkali keerai in Tamil. It is proved that herbal medicine is effective in the treatment of many diseases. The leaves of edible strains are used as food in some locales and plant parts are used as a traditional medicine. Sometimes *Solanum nigrum* is confused for the more toxic deadly nightshade, *Atropa belladonna*, in a different genus altogether. A comparison of the fruit shows that the black nightshade berries grow in bunches; the deadly nightshade berries grow individually (Miraj, 2016) [14].

*Solanum* plants are important source of large number of phytochemical compounds with substantial curative application against human pathogens. It is a wide spectrum of medicinal properties such as anticancer, antioxidant (Al-qirim *et al*., 2008) [1].

Plant growth promoting rhizobacterial (PGPR) association range in degree of bacterial proximity to the root and intimacy of association. In general, these can be separated into extracellular PGPR (EPGPR) existing in the rhizosphere and rhizoplane or in the spaces between cells of the root cortex and intracellular PGPR (IPGPR), which exist inside root cells, generally in specialized nodular structures (Gray and Smith, 2005) [8].

Plant growth promoting rhizobacteria have been reported to directly enhance plant growth by a variety of mechanism fixation of atmospheric nitrogen that is transferred to the plant root, solubilisation of minerals such as phosphorous and synthesis of phytohormones such as auxins (Lucas Garcia *et al*., 2004) [11]. At the present study plant growth promoting rhizobacteria *viz., Azospirillum lipoferum, Azotobacter chroococcum, Bacillus megaterium* and *Pseudomonas fluorescens* were used to increasing Plant growth, chlorophyll and protein content of *Solanum nigrum.*

## 2. Materials methods

### 2.1 Field experiment

The field experiments were conducted backyard of Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar. To study the effect of plant growth promoting rhizobacterial (PGPR) isolates *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens* were used.

### 2.2 Bacterial Strains

The bacterial strains were isolated from rhizosphere soil of *Solanum nigrum*. All the bacterial cultures were isolated used from selective medium *viz.*, *Azospirillium* medium A and B, Waksman base medium, Pikovskaya's and *Pseudomonanas* isolated medium for *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus megaterium* and *Pseudomonas fluorescens* respectively. The isolated stains were stored in refrigerator at 4 ºC for future use.

### 2.3 Treatment details

The treatment details are furnished below.

$T_1$ - *Azospirillum lipoferum* - SAz-9
$T_2$ - *Azotobacter chroococcum* -SAt-1
$T_3$ - *Bacillus megaterium* - SBm-18
$T_4$ - *Pseudomonas fluorescens* - SPf-21
$T_5$ - *Azospirillum lipoferum* - SAz-9+ *Azotobacter chroococcum*- SAt-1
$T_6$ - *Azospirillum lipoferum*- SAz-9 + *Bacillus megaterium* - SBm-18
$T_7$ - *Azospirillum lipoferum* - SAz-9+ *Pseudomonas fluorescens* - SPf-21
$T_8$ - *Azotobacter chroococcum* - SAt-1+ *Bacillus megaterium*- SBm-18
$T_9$ - *Azotobacter chroococcum* - SAt-1+*Pseudomonas fluorescens* - SPf-21
$T_{10}$ - *Bacillus megaterium*-SBm-21+ *Pseudomonas fluorescens*- SPf- 21
$T_{11}$ - Consortium (*Azospirillum lipoferum*- SAz-9+ *Azotobacter chroococcum*–SAt-1+*Bacillus megaterium*- SBm-18 + *Pseudomonas fluorescens* - SPf-21)
$T_{12}$ - Uninoculated control

### 2.4 Biometric observations

From each treatment, three replications were chosen for measuring and recording the biometric observations. Plants were observed at periodic intervals *viz*., 30 DAS, 60 DAS, 90 DAS, 120 DAS and at harvest. The biometric observations like plant height, chlorophyll content and protein content of *Solanum nigrum* were recorded.

### 2.5 Estimation of total chlorophyll content

One gram of fresh leaf sample was taken from each replication for analyzing for total chlorophyll content by following the method of Talling and Driver (1961) [18]. The fresh plant sample was ground with 95 per cent ethanol using pestle and mortar. The extract was allowed to settle in dark for 30 min. The suspension was centrifuged at 5000 rpm at room temperature for 10 min. The supernatant was collected and the volume was made up to a known volume using 95 percent ethanol. The absorbance was read at 665 nm and 649 nm in Elico - Spectrophotometer against 95 per cent ethanol as blank. The total chlorophyll was calculated using the following formula.

Total chlorophyll (mg/g) = 6.10 ($A_{665}$) + 20.04 ($A_{649}$) ×V
Where,
$A_{665}$ - absorbance at 665 nm,
$A_{649}$ - absorbance at 649 nm,
V - Volume of ethanol extract.
The chlorophyll content of leaf sample was estimated and expressed as mg per g fresh weight of sample.

### 2.6 Estimation of protein content

Protein content of leaf sample was determined by the method developed by Bradford (1976) [3].

#### 2.6.1 Bradford's reagent

One hundred mg Coomassie Brilliant Blue G250 was dissolved in 50 ml of 95 per cent ethanol and mixed with 100 ml of concentrated phosphoric acid. The volume was made up to 200 ml with distilled water. The reagent was stored at 4 ºC. This was diluted five times with distilled water prior to use.

#### 2.6.2 Procedure

One gram of fresh leaf sample was extracted with 2 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 5000 rpm for 10 min, at room temperature. To 0.1 ml of clear supernatant, 5.0 ml of Bradford's reagent was added. The blue colour developed was read at 595 nm in Elico-Spectrophotometer. From the standard graph prepared using known quantities of Bovine serum albumin over a concentration ranging from 1to100 µg $ml^{-1}$, the protein concentration of leaf sample was estimated and expressed as mg $g^{-1}$ fresh weight of sample.

## 3. Result

### 3.1 Effect of PGPR inoculation on the plant height (cm) of *Solanum nigrum*

The effect of plant growth promoting rhizobacterial inoculation on plant height in present in (Table -1).
The increased plant height upto atharvest, after sowing was noticed with PGPR inoculation. Among the different treatments, inoculation of PGPR Consortium ($T_{11}$) recorded the maximum plant height of (166.00 cm) on atharvest. The dual inoculant treatment of $T_7$ (161.00 cm), |
$T_9$ (158.00 cm), $T_{10}$ (151.00 cm), $T_5$ (148.00 cm), $T_6$ (147.00 cm), $T_8$ (146.00 cm). The single inoculant treatment of $T_4$ (136.00 cm), $T_1$ (131.00 cm), $T_2$ (129.00 cm), $T_3$ (118.00 cm). The uninoculated control recorded (114.00 cm) and maintains the height at atharvest period of *Solanum nigrum.*

### 3.2 Effect of PGPR inoculation on the chlorophyll content (mg $g^{-1}$) of *Solanum nigrum*

The effect of plant growth promoting rhizobacterial inoculation on the chlorophyll content present in Table - 2.
The chlorophyll content was increased upto 120 days of PGPR inoculation and then gradually decreased with increasing age of the plants. Among the different treatments, inoculation of PGPR Consortium ($T_{11}$) recorded the maximum plant chlorophyll of (5.96 mg $g^{-1}$) on 120 DAS. The dual inoculant treatment of $T_7$ (5.75 mg $g^{-1}$), $T_9$ (5.54 mg $g^{-1}$), $T_{10}$ (4.82 mg $g^{-1}$), $T_5$ (4.67mg $g^{-1}$) and $T_6$ (4.63 mg $g^{-1}$). The single treatment of $T_4$ (4.37 mg $g^{-1}$), $T_1$ (3.91 mg $g^{-1}$), $T_2$ (3.87 mg $g^{-1}$), $T_3$ (3.51 mg $g^{-1}$). The uninoculated control recorded (3.22 mg $g^{-1}$) of *Solanum nigrum.*

### 3.3 Effect of PGPR inoculation on the protein content (mg $g^{-1}$) of *Solanum nigrum*

The effect of plant growth promoting rhizobacterial inoculation on the protein content present in (Table - 3).

The increased protein content was observed upto 120 days of PGPR inoculation and then gradually decreased with increasing age of the plants. Among the different treatments, inoculation of PGPR consortium ($T_{11}$) recorded the maximum plant protein of (13.46 mg $g^{-1}$) on 120 DAS. The dual inoculant treatment of $T_7$ (12.95 mg $g^{-1}$), $T_9$ (12.82 mg $g^{-1}$), $T_{10}$ (12.45 mg $g^{-1}$), $T_5$ (12.42 mg $g^{-1}$), $T_6$ (12.38 mg $g^{-1}$) and $T_8$ (12.25 mg $g^{-1}$).The single treatment of $T_4$ (11.61 mg $g^{-1}$),

$T_1$ (11.27 mg $g^{-1}$), $T_2$ (11.14 mg $g^{-1}$) and $T_3$ (10.81 mg $g^{-1}$). The uninoculated control recorded protein content (10.21 mg $g^{-1}$) in *Solaum nigrum*.

## 4. Discussion

The medicinal plants occupy a significant place in modern medicine as a raw material for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different diseases. The judicious use of medicinal herbs can even cure deadly diseases that have long defied synthetic drugs (Bhattacharjee, 2001) [2].

Co-inoculants of microbes performed better than their individual inoculants. The combination of bacteria interacts with each other synergistically, provide nutrients, remove inhibitory substances and stimulate each other through physical and biochemical activities. Co-inoculation of PGPR with different beneficial properties may be the future trend for biofertilizer application to enable sustainable production (Han and Lee, 2005) [9].

Increase in the height of crop plants due to inoculation of rhizobacteria as observed in the present experiment was in conformity with the earlier reports in other crops (Subba Rao, 1993; Chezian *et al*., 2003) [17, 4].

The chlorophyll and protein content of *Solanum nigrum*, increased due to consortium treatment. The single, dual inoculant effect observed in the present study was in conformity with the earlier reports published on several other crops (Megala and Elango, 2014; Lucy *et al*., 2004; Ghallab and Saleem, 2001; Sivamurugan *et al*., 2000) [13, 12, 7, 16].

## 5. Conclusion

The result of the present study clearly suggested that plant growth promoting rhizobacteria (PGPR) consortium treatment recorded plant height, chlorophyll and protein content of *Solanum nigrum.*

**Table 1:** Effect of plant growth promoting rhizobacteria (PGPR) inoculants on plant height (cm) of *Solanum nigrum*

| SI. No | Treatments | Plant height (cm) | | | | |
|---|---|---|---|---|---|---|
| | | 30DAS | 60DAS | 90DAS | 120DAS | Atharvest |
| 1. | $T_1$ – *A. lipoferum* (SAz-9) | 36.00 | 62.00 | 96.00 | 119.00 | 131.00 |
| 2. | $T_2$ – *A. chroococcum* (SAt-1) | 35.00 | 60.00 | 95.00 | 117.00 | 129.00 |
| 3. | $T_3$ –*B. megaterium* (SBm-18) | 34.00 | 57.00 | 89.00 | 106.00 | 118.00 |
| 4. | $T_4$ – *P. fluorescens* (SPf-21) | 36.00 | 65.00 | 98.00 | 124.00 | 136.00 |
| 5. | $T_5$ – *A. lipoferum* (SAz-9) + *A. chroococcum* (SAt-1) | 40.00 | 70.00 | 110.00 | 138.00 | 148.00 |
| 6. | $T_6$ – *A. lipoferum* (SAz-1) + *B. megaterium* (SBm-18) | 38.00 | 68.00 | 109.00 | 136.00 | 147.00 |
| 7. | $T_7$ – *A. lipoferum* (SAz-9) + *P. fluorescens* (SPf-21) | 42.00 | 78.00 | 122.00 | 152.00 | 161.00 |
| 8. | $T_8$ – *A. chroococcum* (SAt-1) + *B. megaterium* (SBm-18) | 37.00 | 67.00 | 108.00 | 134.00 | 146.00 |
| 9. | $T_9$ – *A. chroococcum* (SAt-1) + *P. fluorescens* (SPf-21) | 42.00 | 77.00 | 121.00 | 147.00 | 158.00 |
| 10. | $T_{10}$ – *B. megaterium* (SBm-18) + *P. fluorescens* (SPf-21) | 41.00 | 72.00 | 113.00 | 141.00 | 151.00 |
| 11. | Consortium $T_{11}$ – *A. lipoperum* (SAz-9) + *A. chroococcum* (SAt-18) +*B. megaterium* (SBm-18) +*P. fluorescens* (SPf-21) | 43.00 | 80.00 | 125.00 | 156.00 | 166.00 |
| 12. | $T_{12}$ – Uninoculated control | 28.00 | 50.00 | 80.00 | 106.00 | 114.00 |
| SED | | 2.36 | 3.85 | 4.01 | 4.69 | 4.31 |
| CD (P= 0.05) | | 4.72 | 7.70 | 8.03 | 9.38 | 8.62 |

**Table 2:** Effect of plant growth promoting rhizobacteria (PGPR) inoculants on total chlorophyll content (mg $g^{-1}$) in *Solanum nigrum*

| SI. No | Treatments | Chlorophyll Content (mg $g^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 30DAS | 60DAS | 90DAS | 120DAS | Atharvest |
| 1. | $T_1$ – *A. lipoferum* (SAz-9) | 2.93 | 3.26 | 3.41 | 3.91 | 3.20 |
| 2. | $T_2$ – *A. chroococcum* (SAt-1) | 2.90 | 3.14 | 3.33 | 3.87 | 3.10 |
| 3. | $T_3$ –*B. megaterium* (SBm-18) | 2.75 | 3.01 | 3.14 | 3.51 | 3.06 |
| 4. | $T_4$ – *P. fluorescens* (SPf-21) | 3.00 | 3.36 | 3.57 | 4.37 | 3.40 |
| 5. | $T_5$ – *A. lipoferum* (SAz-9) + *A. chroococcum* (SAt-1) | 3.80 | 4.11 | 4.26 | 4.67 | 3.90 |
| 6. | $T_6$ – *A. lipoferum* (SAz-1) + *B. megaterium* (SBm-18) | 3.60 | 3.91 | 4.11 | 4.63 | 3.83 |
| 7. | $T_7$ – *A. lipoferum* (SAz-9) + *P. fluorescens* (SPf-21) | 4.30 | 4.70 | 5.01 | 5.75 | 4.80 |
| 8. | $T_8$ – *A. chroococcum* (SAt-1) + *B. megaterium* (SBm-18) | 3.50 | 3.81 | 4.00 | 4.62 | 3.80 |
| 9. | $T_9$ – *A. chroococcum* (SAt-1) + *P. fluorescens* (SPf-21) | 4.25 | 4.60 | 4.81 | 5.54 | 4.53 |
| 10. | $T_{10}$ – *B. megaterium* (SBm-18) + *P. fluorescens* (SPf-21) | 3.90 | 4.14 | 4.37 | 4.82 | 4.10 |
| 11. | Consortium $T_{11}$ – *A. lipoperum* (SAz-9) + *A. chroococcum* (SAt-18) + *B. megaterium* (SBm-18) +*P. fluorescens* (SPf-21) | 4.40 | 4.97 | 5.27 | 5.96 | 5.00 |
| 12. | $T_{12}$ – Uninoculated control | 2.50 | 2.80 | 2.87 | 3.22 | 2.66 |
| SED | | 0.19 | 0.20 | 0.21 | 0.24 | 0.21 |
| CD (P= 0.05) | | 0.38 | 0.40 | 0.42 | 0.49 | 0.42 |

**Table 3:** Effect of plant growth promoting rhizobacteria (PGPR) inoculants on total protein (mg $g^1$) in *Solanum nigrum*

| SI. No | Treatments | Protein content (mg $g^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | 30DAS | 60DAS | 90DAS | 120DAS | Atharvest |
| 1. | $T_1$ – *A. lipoferum* (SAz-9) | 10.48 | 10.63 | 10.95 | 11.27 | 11.00 |
| 2. | $T_2$ – *A. chroococcum* (SAt-1) | 10.21 | 10.51 | 10.84 | 11.14 | 10.90 |
| 3. | $T_3$ –*B. megaterium* (SBm-18) | 9.85 | 10.12 | 10.47 | 10.81 | 10.53 |
| 4. | $T_4$ – *P. fluorescens* (SPf-21) | 10.67 | 10.93 | 11.21 | 11.61 | 11.30 |
| 5. | $T_5$ – *A. lipoferum* (SAz-9) + *A. chroococcum* (SAt-1) | 11.13 | 11.77 | 12.02 | 12.42 | 12.07 |
| 6. | $T_6$ – *A. lipoferum* (SAz-1) + *B. megaterium* (SBm-18) | 11.12 | 11.68 | 11.99 | 12.38 | 11.96 |
| 7. | $T_7$ – *A. lipoferum* (SAz-9) + *P. fluorescens* (SPf-21) | 11.52 | 12.01 | 12.35 | 12.95 | 12.50 |
| 8. | $T_8$ – *A. chroococcum* (SAt-1) + *B. megaterium* (SBm-18) | 10.90 | 11.20 | 11.51 | 12.25 | 11.96 |
| 9. | $T_9$ – *A. chroococcum* (SAt-1) + *P. fluorescens* (SPf-21) | 11.40 | 11.96 | 12.21 | 12.82 | 12.40 |
| 10. | $T_{10}$ – *B. megaterium* (SBm-18) + *P. fluorescens* (SPf-21) | 11.15 | 11.51 | 11.81 | 12.45 | 12.10 |
| 11. | Consortium $T_{11}$ – *A. lipoperum* (SAz-9) + *A. chroococcum* (SAt-18) +*B. megaterium* (SBm-18) +*P. fluorescens* (SPf-21) | 12.01 | 12.36 | 12.62 | 13.46 | 12.96 |
| 12. | $T_{12}$ – Uninoculated control | 9.30 | 9.50 | 9.73 | 10.21 | 9.96 |
| SED | | 0.119 | 0.169 | 0.179 | 0.223 | 0.144 |
| CD (P = 0.05) | | 0.239 | 0.339 | 0.359 | 0.450 | 0.289 |

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