**INTRODUCTION**

PGPRs are a diverse group of soil bacteria that inhabit rhizosphere, around/on the root surface, that can improve plant growth directly or indirectly mediated by a variety of mechanisms. PGPR are widely considered as potential tools for sustainable agriculture that can influence plant growth and development either by releasing phytohormones or other biologically active substances, altering endogenous levels of plant growth regulators (PGR), enhancing availability and uptake of nutrients through fixation and mobilization, reducing harmful effects of pathogenic microorganisms on plants and/or employing multiple mechanisms of action (Parewa, H. P. et al., 2018). Significant improvement in growth and productivity of agricultural crops in response to PGPRs under greenhouse, field as well as stress conditions has been reported by several authors (Arshad, M. et al., 2008; Mubeen, F. et al., 2008; &Naiman, A. D. et al., 2009). PGPRs are mostly used as seed, soil or foliar inoculants for improving the growth and productivity of agricultural crops. In the longer run they have been used as components in biofertilizer technology to improve the efficiency of agricultural crops (Naiman, A. D. et al., 2009). These microbes interact with plants in the rhizospheric region and can influence plant growth by one or a combination of several response mechanisms, of which foremost importance for plant growth as well as for soil health is held by nutrient cycling in soil and ecosystem (Babalola, O. O. 2010). PGPRs can also alter root architecture and promote plant development with the production of varied phytohormones like IAA, gibberellic acid and cytokinins (Kloepper, J. W. et al., 2007).

Inoculation with PGPRs strains could significantly improve plant growth and development under contaminated agroclimatic conditions (Denton, B. 2007). One of the most used and reported organisms belong to the class of Fluorescent pseudomonads and Bacillus species that exhibit very high efficiency in host root colonization and production of growth metabolites resulting in improved strategic crop yield (Khalid, A. et al., 2004). (Swain, M. R. et al., 2007) reported a positive effect of IAA producing strains of Bacillus subtilis on Dioscorea rotundata L. They applied a suspension of B. subtilis on the surface of the plant, which resulted in an increase in the root:stem ratio as compared with the non-inoculated plants. Some Pseudomonas species also improve the plant growth through the production of water-soluble vitamins like niacin. PGPRs can also work as biocontrol agents providing protection to the plants, enhancing the plant growth through the synthesis of antibiotics (Riaz, U. et al., 2021). Considering the facts presented above the present investigation was planned to evaluate the effect of PGPRs on growth and biochemical properties of the radish plants and the results obtained are presented below.
MATERIALS AND METHODS:

- **Bacteria Isolates:**
  Four standard PGPR strains viz. *Pseudomonas putida, Pseudomonas fluorescens, Bacillus subtilis* and *Azospirillum lipoferum* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India.

- **Plant Material and Treatment:**
  Radish seeds, were obtained from the Vegetable Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, India. The seeds were sown and allowed to grow up to three leaf stage before the bacterial treatment was given. For treatment the cultures obtained from MTCC was activated and multiplied in Luria broth. When bacteria obtained abundant population (10^6 to 10^7 cfu) they were applied to Radish plants. The plant population was maintained as treated and untreated control plants. At 10 and 20 days after treatment (DAT) different parameters like plant height, number of leaves, NR activity, SOD activity and proteins content were recorded. After harvesting fresh weight and dry weight of plants were recorded.

- **Nitrate Reductase (NR) Activity:**
  The NR activity was estimated in vivo in the freshly harvested leaves by using the method described by (Hageman, R. H., & Hucklesby, D. P. 1971). To estimate the NR activity, 0.5 g of freshly harvested leaf tissue was cut into pieces and transferred into a test-tube containing 3 ml each of pre-chilled KNO3 and phosphate buffer. It was then incubated in dark at 33^º C. Aliquot of 0.2 ml twice at 10 min and 40 min after incubation was taken in separate test tubes containing 1 ml distilled water. Add 2 ml [1:1 (v:v)] mixture of NEDD and sulfanilamide and kept in test tube in dark for about 15 min for pink color development. Then absorbance was measured at 540 nm with the help of spectrophotometer.

- **Superoxide Dismutase (SOD) Activity:**
  The superoxide dismutase activity (SOD activity) was estimated in vitro in the freshly harvested leaves by using the method described by (Gianopolitis and Reis, 1969). The enzyme was extracted by homogenizing 0.2 g leaf sample in 4 ml extraction buffer (100 mM phosphate buffer and 0.1 mM EDTA) followed by centrifugation at 10,000 rpm for 15 minutes. For measurement of enzyme activity 100 µl of enzyme extract was added into 3.0 ml reaction mixture (50 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM NBT and 2 µM riboflavin) and the tubes were illuminated with fluorescent tubes. The reaction was allowed to proceed for 15 minutes and the absorbance was recorded at 560 nm.

- **Protein Content:**
  Protein was estimated from leaves of Radish plant material by using the method described by (Bradford, M. M. 1976). For extraction 0.2 gm leaves were homogenized in tris-buffer and the filtrate was centrifuged at 10,000 rpm for 15 minutes to get the protein extract. For estimation 0.2 ml supernatant was mixed with 0.8 ml distilled water to which 3 ml Bradford dye was added. The absorbance of the resulting mixture was recorded at 595 nm. Protein content was calculated from standard curve prepared from BSA.

- **Statistical Analysis:**
  Significant differences between the effects produced by different PGPR inoculants were shown by one-way ANOVA in accordance with the experimental design (strip plot design) using SPSS-16 statistical package.

RESULTS AND DISCUSSION:

- **Plant Morphology:**
  - **Plant Height:**
    Plant height is an important parameter that tells us about the vegetative growth of the plants. In case of radish more plant health means more the leaf surface available to interrupt the available sunlight. The data obtained for plant height (fig 1) showed that different treatments were effective in increasing the plant height in comparison with uninoculated control. At 10 and 20 DAT maximum increase in plant height was observed in plants treated with *Bacillus subtilis* with an increase of 12.98 % and 12.7% respectively in comparison to the control plants. Similar results were demonstrated by (Nezarat, S., & Gholami, A, 2009), who reported that inoculation of maize seeds with different bacterial strains viz. *Pseudomonas putida, Pseudomonas fluorescens, Azospirillum lipoferum, Azospirillum brasilense* significantly increased the plant height (14.3-21.7%). Similar increases in plant height were observed in different crops such as Potato, Radish plants, Sorghum and Pearl millet inoculated with *Pseudomonas, Azospirillum and Azotobacter* strains (Kamran, S. et al., 2010).

![Figure 1: Plant height (cm) of Radish in treated and control plants. The values presented are mean of 5 rows of 5 replicates each.](image)

- **Leaf Number:**
  Number of leaves is a parameter that is directly related to the productivity of plants in respect to the fact
that more number of leaves means higher surface area and higher will be the amount of sunlight intercepted by the plants. The data was obtained (fig 2) showed that leaf number was higher in the treated plants in comparison to the control plants. At 10 DAT maximum number of leaves was recorded for *Pseudomonas fluorescens* with an increase of 11.48 % in comparison to control, whereas at 20 DAT maximum number of leaves was noted for *Pseudomonas putida* with an increase of 11.22 % in comparison to the untreated control plants. Similar results were reported by (Sen, S., & Chandrasekhar, C. N. 2014), who reported an increase in the leaf number of rice plants treated with *Pseudomonas* strain TDK1.

![Figure 2: Number of leaves of Radishes in treated and control plants. The treatments were given at 3 leaf stage and the observations were recorded 10 days later. The values presented are mean of 5 rows of 5 replicates each.](image)

**Fresh Weight:**

Plant fresh weight after harvesting was measured to determine the effect of application of PGPRs on the direct growth of the plants and is presented in figure 3. Highest fresh weight was recorded in plants treated with *Pseudomonas putida* showing an increase of almost 43.56% in comparison to the control plants. But as roots being the economically important part of the plants their fresh weight was taken separately where maximum fresh weight was observed for plants treated with *B. subtilis* showing an increase of 41% over control plants and leaves weighed alone showed an increase of 51% in the plants treated with *P. putida* when compared to the control plants. Similar results were shown by (Veresoglou, S. D., & Menexes, G. 2010) who argued that inoculation with *Azospirillum* increases the production of dry biomass in plants by up to 33%, as indole acetic acid synthesized by the bacteria is used by the plant.

![Figure 3: Fresh weight (g) of Radishes in treated and control plants. The values presented are mean of 5 rows of 5 replicates each.](image)

**Dry Weight:**

Dry Weight of oven dried radish plants was measured to evaluate the effect of PGPR application on biomass accumulation and the data is presented in figure 4. Data of dry weight was recorded for complete plants and also for roots and leaves distinctly. Plant, root and leaf dry weight was found to be highest for the plants treated with *P. putida* showing an increase of 47.72 % in case of plant dry weight, 50% in root dry weight and almost 42% in case of leaf dry weight when compared to the untreated control plants. Similar results were shown by (Lomonosova, V. A. et al., 2020), who used a commercial preparation Korne plus based on the bacterium *Pseudomonas putida* K-9. Application of this preparation resulted in the yield of radish (1.98 ± 0.22 kg) which was significantly higher than in the control (1.52 ± 0.19 kg). The yield increase was 0.46 kg / m2 (30.3 %).

![Figure 4: Dry weight (g) of Radishes in treated and control plants. The treatments were given at 3 leaf stage and the observations were recorded at harvesting stage of plants. The values presented are mean of 5 rows of 5 replicates each.](image)
Table 1: Dry weight (g) of Radish Plants treated with different bacterial inoculants and untreated control plants after harvesting.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Complete plants</th>
<th>Roots</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas putida</td>
<td>230.34±97.13</td>
<td>165.57±78.42</td>
<td>64.8±23.85</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>213.58±17.86</td>
<td>149.44±31.28</td>
<td>63.5±17.76</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>150.85±17.94</td>
<td>103.89±15.8</td>
<td>47.08±12.69</td>
</tr>
<tr>
<td>Azospirillum lipoferum</td>
<td>172.18±30.46</td>
<td>110.49±23.19</td>
<td>61.18±19.96</td>
</tr>
<tr>
<td>Control</td>
<td>120.42±20.56</td>
<td>82.67±11.24</td>
<td>37.66±8.22</td>
</tr>
</tbody>
</table>

**Biochemical Parameters:**

- **Nitrate Reductase Activity:**
  
  NR activity is an important parameter that can give us an idea regarding the assimilation of nitrate and plant growth. In the present investigation NR activity was measured and the data obtained is presented in figure 5. During the planting season all the treatments showed an improvement in NR activity in comparison to the control plants. At 7 DAT maximum NR activity was recorded in the Radish plants treated with *P. fluorescens* with an increase of almost 43% in comparison to control plants. At 14 DAT plants treated with *B. subtilis* with an increase of 40% and at 21 DAT plants treated with *A. lipoferum* showed highest NR activity with an increase of 47% in comparison to the untreated control plants. Similar results were shown by (Lee, S. et al., 2020) who examined the effect of *Bacillus subtilis* strain L1 (Bs L1) on NR activity of plants. They showed that plants treated with PGPR had higher NR activity than control in Wheat and Lettuce plants. These results suggest that Bs L1 promotes the assimilation and use of nitrate and plant growth.

- **Superoxide Dismutase (SOD) Activity:**

  SOD activity was measured at 7, 14 and 21 days after the treatment in the treated and untreated radish plants and the data obtained is presented in figure 6. During the planting season at 7 DAT highest SOD activity was recorded for *A. lipoferum* showing an increase of 59% in comparison to the control plants. However at 14 DAT highest SOD activity was recorded for *P. fluorescens* with an increase of 51.61% in SOD activity when compared with control plants, and at 21 DAT plants treated with *B. subtilis* showed highest SOD activity showing an increase of 67.24 % in comparison to the untreated control plants. Similar results were demonstrated by (Jha, Y., & Subramanian, R. B. 2013) who reported that plants inoculated with PGPR showed increased SOD activity that ranged from 7% to 69% at different salinity levels as compared to plants under non-saline conditions in paddy.

- **Protein Content:**

  Protein content was determined in the radish plants in response to inoculation with different PGPR strains and the data obtained is presented in the figure 7. At 7 DAT maximum protein content was found in plants treated with *P. putida* that showed an increase of almost 2.6 % in comparison to the untreated control plants. While at 14 DAT maximum protein content was found in plants treated with *P. fluorescens* showing an increase of 13% and at 21 DAT highest protein content was
found in plants treated with *P. fluorescens* showing an increase of 3% in comparison to the untreated control plants. It has been reported that inoculation with bacterial isolates can increase the protein content plants by (Ilyaset al., 2020). They reported that bacterial inoculation increased protein synthesis in plants. Among inoculated plants, combination-treated plants showed better results with 46.82% and 42.45% increase in protein content as compared to untreated plants. They used the bacterial isolates *A. brasilense* and *B. subtilis* for their experimentation with wheat plants.

![Graph](image)

**Figure 7:** Protein content (mg g⁻¹ FW) in leaves of Radish in treated and Control plants. The treatments were given at 3 leaf stage and the observations were recorded 7, 14 and 21 days later. The values presented are mean of 3 replicates each.

**CONCLUSION:**

Use and application PGPRs in sustainable agriculture has been of paramount importance; it has been proven to be an environmentally sound way of increasing crop yields through either direct or indirect mechanism including regulating hormonal and nutritional balance. In conclusion, it was found that four plant growth promoting rhizobacteria viz. *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Azospirillum lipoferum* application in rhizosphere of radish plants significantly enhanced the growth, root and shoot length and biomass production of Radish. Therefore it is suggested that the use of PGPR isolates as biofertilizers is an important strategy and can prove to be beneficial for Radish cultivation.

**REFERENCES:**

rhizospheric microbes in soil (pp. 299-329). Springer, Singapore.


