Effects of PGPR (Pseudomonas sp.) and Ag-nanoparticles on Enzymatic Activity and Physiology of Cucumber

Sehrish Nawaz¹ and Asghari Bano¹,*

¹Department of Biosciences, University of Wah, Wah Cantt, Pakistan

Abstract: Background: The present investigation aimed to evaluate the role of Plant Growth-Promoting Rhizobacteria (PGPR) and Ag-nanoparticles on two varieties (American variety, Poinsett 76 and Desi variety, Sialkot selection) of cucumber plants.

Methods: Cucumber seeds prior to sowing, were inoculated with two strains of PGPR, Pseudomonas putida (KX574857) and Pseudomonas stutzeri (KX574858) at the rate of 10⁶ cells/ml. Ag-nanoparticles (5ppm) were sprayed on the plant at early vegetative phase 27 d after sowing.

Results: The proline, sugar, protein, phenolics, flavonoids, chlorophyll and carotenoids contents of leaves of plants and the activities of Phenylalanine Ammonia-Lyase (PAL), Superoxide Dismutase (SOD) and Catalase (CAT) were determined from leaves of plants at early vegetative phase. After 3 months of seeds sowing, Ag-nanoparticles enhanced the length of root but decreased the length of shoot and fresh weight of root and shoot as compared to control whereas, the leaf protein, proline, phenolics, flavonoids, chlorophyll b, total chlorophyll, sugar and Phenylalanine Ammonia-Lyase (PAL) activity of plants were increased significantly over control. Ag-nanoparticles also suppressed the effect of PGPR for root, shoot length but augmented the protein and phenolics contents of leaves of both the varieties.

Conclusion: The combined treatment of Ag-nanoparticles and PGPR enhanced flavonoids content of leaves and the activities of PAL, SOD and CAT in leaves of plants over control. Ag-nanoparticles effectively increased the Phenylalanine Ammonia-Lyase (PAL), Catalase (CAT) and superoxide dismutase (SOD) activities in leaves of both the varieties. Pseudomonas putida may be used either alone or in combination with Ag-nanoparticles to enhance the antioxidant and defense enzyme activities. Hence, the plant can tolerate the diseases and stresses in a much better way with higher protein and phenolics content.

Keywords: Ag-nanoparticles, PGPR, cucumber, antioxidant enzymes, PAL, carotenoids.

1. INTRODUCTION

Cucumis sativus L. commonly known as Khira is abundantly used as salad [1]. It has several important nutrients Fe, Ca, Mg, S, P, K, Na, Mn, Zn and also phenolic compounds (caffeic, p-coumaric acid, ferulic acids and p-coumaric acid methylester). The compounds act as protective scavengers against Reactive Oxygen Species (ROS) and oxygen-derived free radicals that play an important role in the prevention of cardiovascular disease and also had anticancer activity [2]. It contains low calories and high amount of water. It has antioxidant activity, lowers lipid content and had antidiabetic potential. Cucumber is used to prevent constipation and has a cooling effect on the body [3].

Silver Nanoparticles (AgNPs) are currently one of the most broadly used nanomaterials [4]. Silver or silver ions have a strong effect on inhibition and killing of bacteria and exhibit the anti-microbial activity. The silver nanoparticle has the ability to eliminate plant diseases and pests by discharging Ag⁺ ions [5]. The effect of Ag-nanoparticle alone had already been studied on Jasmine rice (Oryza sativa. L) to improve the seed germination and growth of seedling [6].

Plant Growth-Promoting Rhizobacteria (PGPR) are native to plant rhizosphere and also act as a biocontrol agent against plant pathogens [7]. PGPR promote the production of phytohormones and other signals that enhance the lateral root branches and the development of root hairs. PGPR modify the function of roots, improve the nutrition of plants and enhance the physiology of plants [8]. Seed germination rate, root development, root and shoot weights of leaf and chlorophyll and protein contents and nutrient uptake are provided with PGPR [9]. They play an important role in nitrogen fixation [10, 11].

Plant Growth Promoting Rhizobacteria (PGPR) improved the growth of plants. Different strains of PGPR are used to control the fungal, viral and bacterial diseases [12]. Two of
the most important biocontrol agents are a member of the genera Bacillus and Pseudomonas. Both bacterial agents have Plant Growth-Promoting (PGP) properties [13]. PGPR strains induce systemic resistance in plants through the activation of different defence-related enzymes such as chitinases, Peroxidase (PO), Polyphenoloxidase (PPO), Phenylalanine Ammonia-Lyase (PAL) and β-1, 3-glucanase [14].

The effect of Ag-nanoparticle alone has already been studied on the cucumber and pumpkin to inhibit the powdery mildews [15]. AgNPs has the bactericidal and fungicidal effect and showed positive and negative effects on root growth, germination of seed and plant biomass [16].

The present investigation was aimed to improve the growth of cucumber plants by the application of Plant Growth Promoting Rhizobacteria (PGPR) and Ag-nanoparticles.

2. MATERIALS AND METHODS

2.1. Sterilization of Seeds

The two varieties of seeds of cucumber Desi (Sialkot selection) and American (Poinsett 76) were sterilized in 95% ethanol for 3-4 min; the seeds were soaked in 10% chlorox with shaking for 2-3 min and subsequently washed 3-4 times with the autoclaved distilled water.

2.2. Preparation of Bacterial Inocula

Two strains of PGPR Pseudomonas putida (KX574857) and Pseudomonas stutzeri (KX574858) were used. The LB media was inoculated with 24 h old culture and incubated in shaking incubator at 30°C for 72 hours. The inocula was centrifuged at 3000 rpm for 10 min, the supernatant was discarded while the pellet was suspended in distilled water to adjust the optical density 1 at 660 nm which was equivalent to 10⁶ cell per ml. Then sterilized seeds were soaked in bacterial inocula for 2-3h whereas, control sterilized seeds were soaked in LB broth for 2-3h. The seeds were sown in pots (6-7 inches) filled with soil and sand (3:1) mixed with cow dung. Seeds were then allowed to grow under natural conditions. The Ag nanoparticles was donated by Hussain and Mahmood [17]. After 27d of seeds germination, the plants' leaves were sprayed with silver-nanoparticles (5ppm).

There were 5 pots/ treatment. V1= American variety (Poinsett 76), V1C1= Untreated plants of American variety (treated as control), V1N= plants sprayed with 5ppm Ag-nanoparticles, V1P1= Seeds inoculated with Pseudomonas putida, V1P2= Seeds inoculated with Pseudomonas putida and plants sprayed with 5ppm Ag-nanoparticles, V1NP1= Seeds inoculated with Pseudomonas putida and plants sprayed with 5ppm Ag-nanoparticles. V2= Desi variety (Sialkot selection), V2C2= Untreated plants of Desi variety (treated as control), V1N= plants sprayed with 5ppm Ag-nanoparticles, V1P1= Seeds inoculated with Pseudomonas putida, V1P2= Seeds inoculated with Pseudomonas putida, V1NP1= Seeds inoculated with Pseudomonas putida and plants sprayed with 5ppm Ag-nanoparticles, V1NP2= Seeds inoculated with Pseudomonas stutzeri and plants sprayed with 5ppm silver-nanoparticles.

2.3. Physiological and Biochemical Analyses of Plants

After 9 weeks of seed germination, the number of branches and flowers were counted. Length and weight of shoot and root were measured after 3 months of seed germination.

2.4. Biochemical Analysis of Plants

2.4.1. Chlorophyll and Carotenoids Content of Leaves

Chlorophyll and carotenoids contents were measured following the method of Khan et al. [18]. Fresh leaves (100 mg) were homogenized in 5ml of 80% acetone, incubated for 5 min at 90°C in the water bath. The extracts were centrifuged at 3000 rpm for 10 min. The OD of supernatant was recorded for chlorophyll a, b and carotenoids contents at 663, 445 and 480 nm against 80% acetone blank, respectively.

Chlorophyll a = 12.7 x A663 - 2.69 x A645
Chlorophyll b = 22.9 x A645 - 4.68 x A663
Total chlorophyll = (12.7 x A663) + (22.9 x A645)
Carotenoids = 4 × OD × total sample vol. / fresh weight of cucumber leaves.

2.4.2. Sugar Content

The sugar content of leaves of plants was determined following the method of Dubois [19] using glucose as standard. Fresh plant material (0.5 g) was homogenized in 10 ml of distilled water in a clean mortar and pestle and centrifuged at 3000 rpm for 15 mins. 1 ml of 0.5% (v/v) phenol was added in 0.1 ml of supernatant. Incubation was done at room temp for 1 h. Then 5 ml of concentrated H₂SO₄ was added. The absorbance of each sample was recorded at 420 nm against blank.

2.4.3. Protein Content

The protein content of fresh leaves was determined following the method of Khan et al. [18]. Fresh leaves (0.1 g) were grinded with the help of mortar and pestle in 1 ml of phosphate buffer and centrifuged for 10 min at 3000 rpm. The supernatant (0.1 ml) was poured in a test tube and total volume of 1 ml was made by distilled water. Reagent C (1ml) was added. After shaking for 10 minutes, 0.1 ml of reagent D was added. The absorbance of each sample was recorded at 650 nm after 30 minutes incubation. The concentration of protein was determined by the following formula:

Protein content mg/g = K value × Dilution Factor × Absorbance/ Weight of sample

K value = 19.6

2.4.4. Proline Content

The proline content of leaves was determined following the method of Bates [20]. Leaves were homogenized in 5 ml of 3% sulphosalycylic acid by using the mortar and pestle. The extracts were centrifuged at 3000 rpm for 15 min. 2 ml of the supernatant was taken in the test tube, then 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent was added. The reaction mixture was incubated at 100°C for 1 h. The reaction mixture was cooled and 4ml of the toluene was added. Brick red color appeared. After thorough mixing, the toluene layer was separated. The upper layer of the reaction
methanol. Water (400 µl) and 1ml of AlCl₃ reagent were crystalline sodium acetate, dissolved in 100 ml of 80% Total flavonoids were determined using AlCl₃ method of 2.4.5. Flavonoids Content

Total flavonoids were determined using AlCl₃ method of Zhishen [21]. The homogenate prepared in 80% methanol were centrifuged at 3000 rpm for 10 min. The AlCl₃ reagent was prepared by taking 133 mg crystalline AlCl₃ and 400 mg crystalline sodium acetate, dissolved in 100 ml of 80% methanol. Water (400 µl) and 1ml of AlCl₃ reagent were added to 2 ml of supernatant. After thorough mixing, the absorbance was recorded at 430 nm against blank. The total flavonoids content was expressed as mg quercetin equivalent per gram of extract (mg QE/g).

2.4.6. Phenolics Content

Phenolics content of leaves was determined by using the Folin-Ciocalteu method with Gallic acid as standard Singleton and Jones [22]. Plant extract (1 ml) was mixed with 9 ml distilled water and 1 ml Folin Ciocalteu reagent, 10 ml sodium-carbonate (7%) added to the mixture. The mixture was incubated for 90 min at room temperature, absorbance was recorded at 765 nm. The total phenolic content was expressed as mg gallic acid equivalent per gram of extract (mg GAE/g).

2.5. Enzymes Assays

2.5.1. Phenylalanine Ammonia-lyase (PAL)

Phenylalanine ammonia-lyase (PAL) activity was determined following the method of Suzuki [23] and Dos Santos [24]. Fresh leaves (1 g) were grounded at 4°C in 5 ml of 0.1 M sodium borate buffer (pH 8.8). Then extract was centrifuged at 4°C (12,000 × g for 15 min) and the supernatant was used as the enzyme extract. The reaction mixtures consist of 500µL sodium borate buffer (pH 8.7) and 250 µL enzyme extracts pre-incubated at 40°C (5 min) and the reaction will start by adding 300µL of 50 mM L-phenylalanine. After 1 h incubation at 40°C, the reaction was stopped by adding 50 µL of 5 N HCl. The reaction mixture was centrifuged again at 3000rpm for 15 min and absorbance recorded at 275 nm. The activity of PAL was expressed as; nmol L-cinnamate min⁻¹ g⁻¹ of fresh mass in relation to the peak area of L-CA standard solution (1 mg 100 mL⁻¹ sodium borate buffer pH 8.7).

2.5.2. Superoxide Dismutase (SOD)

Superoxide Dismutase (SOD) was determined following the method of Beauchamp and Fridovich [25]. Fresh leaves (0.2 g) were grounded in 4ml phosphate buffer (PH7.8), containing 1% PVP centrifuged at 15000 g for 15 min at 4°C. The supernatant about 0.8 ml was collected in a test tube. The reaction mixture contains riboflavin (1.17 × 10⁻⁶), methionine (0.1M), potassium cyanide (2 × 10⁻⁵) and nitroblue tetrazolium (5.6 × 10⁻⁵) dissolved in 3 ml 0.05 M sodium phosphate buffer. The reaction mixture (3 ml) was mixed with 1 ml of enzyme extract. To initiate the reaction, one sample was kept in light at 30°C for 1 h and the same sample was kept in dark that served as blank. The absorbance was recorded at 560nm.

2.5.3. Catalase Activity (CAT)

Catalase activity was performed by using the method of Kumar [26] with some modifications. Enzyme extract 0.2 ml was taken and to make the reaction mixture, 3% of 0.1 mL H₂O₂ and 0.5 M of 0.5 mL potassium phosphate buffer (pH 7) were added in the supernatant of extract. The absorbance was measured at 240 nm at 0 min and 3 min. Catalase activity (units/mg protein/min - Decrease in absorbance (initial reading at 0min – final reading at 3 min) × 100 ÷ protein amount (mg)).

3. RESULTS

The mean, standard deviation and standard error have been calculated using three replicate per treatment by using the software graph pad prism 6.

3.1. Number of Branches

In V1, there was no significant increase in the number of branches except in V1N (plants sprayed with Ag-nanoparticles) plants (Table 1) which showed (5%) increase as compared to control V1C1 plants. There was a 26% decrease in the number of branches in V1P2 plants (seed treated with *Pseudomonas stutzeri*) as compared to control V1C1.

In V2, there was no significant effect of the treatments except in V2N and V2P1 plants as compared to control V2C2. The maximum increase in the number of branches was 19% in V2N plants (plants treated with Ag-nanoparticles).

3.2. Number of Flowers

In V1, the number of flowers was increased in all the treatments except in V1NP1 plants (Table 1). The maximum increase was 49% in V1P2 plants (seeds treated with *Pseudomonas stutzeri*) as compared to control V1C1. The number of flowers was 50% less in Sialkot selection than that of Poinsett 76.

In V2, there was 27% increase in the number of flowers in V2NP2 plants (seeds treated with *Pseudomonas stutzeri*) and plants sprayed with 5ppm Ag-nanoparticles). There was 26% decrease in the number of flowers in V2P1 plants (seeds treated with *Pseudomonas putida*).

3.3. Length of Shoot

In V1, the length of shoot was decreased in all the treatments except in V1P1 and V1NP1 (Table 2). The maximum increase in the length of shoot was 20% in V1P1 (seeds treated with *Pseudomonas putida*). There was 26% decrease in the length of shoot in V1NP2 (combined treatment with *Pseudomonas stutzeri* and Ag-nanoparticles) than that of control V1C1.

The length of shoot was decreased in all the treatments in V2 except in V2P1. The maximum increase in the length of shoot was 3.45% in V2P1 (seeds treated with *Pseudomonas putida*). The maximum decrease in the length of shoot was
54% in V2N (plants sprayed with Ag-nanoparticles) as compared to control V2C2.

3.4. Length of Root

In V1, the length of the root was increased in all the treatments (Table 2). The maximum increase in the length of root was 30% in V1N (plants sprayed with Ag-nanoparticles) as compared to control V1C1.

In V2, the length of root was decreased in all the treatments except in V2N (Ag-nanoparticles treatments) plants showing a maximum increase of 79% in the length of root as compared to control V2C2.
compared to control V2C2. The maximum decrease in the length of root was 54% in V2P1 (seeds treated with *Pseudomonas putida*) as compared to control V2C2.

### 3.5. Weight of Shoot

The data in Table 3 indicated that in V1, the weight of fresh shoot was increased in all the treatments except in V1N and V1NP2 plants. The maximum increase was 63% in V1P1 plants (seeds treated with *Pseudomonas putida*).

In V2, the fresh weight of shoot was decreased in all the treatments. The maximum decrease was 59% in V2NP1 (seeds treated with *Pseudomonas putida* and plants sprayed with Ag-nanoparticles) as compared to control V2C2.

### 3.6. Weight of Root

In V1, the fresh root weight was increased in all the treatments (Table 3). The maximum increase was 48% in V1P2 (seeds treated with *Pseudomonas stutzeri*) as compared to control V1C1.

In V2, there was no significant effect on the fresh root weight except in V2N plants. There was 48% increase in the fresh root weight in V2N (plants sprayed with Ag-nanoparticles) as compared to control.

### 3.7. Protein Content

Fig (1) showed that the protein content in V1 was increased in all the treatments except in V1NP2. The maximum increase in the protein content was 51% in V1P1 (Treatment with *Pseudomonas putida*). There was 6.27% decrease in the protein content in V1NP2 (Treatment with *Pseudomonas stutzeri* and plants sprayed with Ag-nanoparticles) than that of control V1C1.

In V2, protein content was increased in all the treatments except in V2NP2. The maximum increase in protein content was 53% in V2N (treated with Ag-nanoparticles). There was a 5% decrease in protein content in V2NP2 (combined treatment with *Pseudomonas stutzeri* and Ag-nanoparticles) as compared to V2C2.

### 3.8. Proline Content

Fig. (2) revealed that in V1, proline content was augmented in all the treatments. The maximum increase was 25% in V1P1 (seeds treated with *Pseudomonas putida*) than that of control.

In V2, the proline content in V2 was also higher than control in all the treatments except in V2NP1. The maximum increase in proline content was 44% in V2P1 (seeds treated with *Pseudomonas putida*).

### 3.9. Phenolics Content

In V1, the phenolics content was increased in all the treatments (Fig. 3). The maximum phenolics content was 31% in V1NP1 (combined treatment with *Pseudomonas putida* and Ag-nanoparticles).

In V2, all the treatments responded positively and the phenolics content was increased as compared to V2C2. The maximum phenolics content was 33% in V2NP1 (combined treatment with *Pseudomonas putida* and Ag-nanoparticles) and 32% in V2NP2 (combined treatment with *Pseudomonas stutzeri* and Ag-nanoparticles).

### 3.10. Flavonoids Content

Fig. (4) indicated that in V1, the flavonoids content was increased in all the treatments except in V1P1 plants which showed no significant decrease of 3% than that of V1C1. The maximum flavonoids content was 48% in V1P2 (combined treatment with *Pseudomonas stutzeri* and Ag-nanoparticles) than that of V1C1.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Fresh Shoot Weight (g)</th>
<th>Fresh Root Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1C1</td>
<td>18.13±2.02</td>
<td>1.63±0.11</td>
</tr>
<tr>
<td>V1N</td>
<td>16.27±1.07</td>
<td>1.82±0.27</td>
</tr>
<tr>
<td>V1P1</td>
<td>29.5±±2.64</td>
<td>2.45±0.26</td>
</tr>
<tr>
<td>V1P2</td>
<td>23.38±9.01</td>
<td>3.34±0.35</td>
</tr>
<tr>
<td>V1NP1</td>
<td>21.86±4.79</td>
<td>2.54±0.17</td>
</tr>
<tr>
<td>V1NP2</td>
<td>15.32±2.13</td>
<td>2.03±0.33</td>
</tr>
<tr>
<td>V2C2</td>
<td>36.66±2.78</td>
<td>3.06±0.51</td>
</tr>
<tr>
<td>V2N</td>
<td>24.31±5.41</td>
<td>4.52±0.63</td>
</tr>
<tr>
<td>V2P1</td>
<td>31.07±1.24</td>
<td>3.37±0.19</td>
</tr>
<tr>
<td>V2P2</td>
<td>29.3±3.83</td>
<td>3.79±0.23</td>
</tr>
<tr>
<td>V2NP1</td>
<td>14.72±0.56</td>
<td>2.32±0.46</td>
</tr>
<tr>
<td>V2NP2</td>
<td>19.71±1.65</td>
<td>2.33±0.06</td>
</tr>
</tbody>
</table>

The data represents mean of 3 replicates and ± represents standard error value. Treatments detail as shown in Table 1.
**Fig. (1).** Effects of PGPR and Ag-nanoparticles on protein content of leaves of cucumber plant. The values are the mean of three replicates. The bar on graph represented standard error value. V1= American variety (Poinsett 76), V1C1= Untreated control plants of American variety (Poinsett 76), V1N= Plants sprayed with 5ppm Ag-nanoparticles, V1P1= Seeds treated with *Pseudomonas putida*, V1P2= Seeds treated with *Pseudomonas stutzeri*, V1NP1= Seeds treated with *Pseudomonas putida* and plants sprayed with 5ppm Ag-nanoparticles, V1NP2= Seeds treated with *Pseudomonas stutzeri* and plants sprayed with 5ppm Ag-nanoparticles. V2= Desi variety (Sialkot selection), V2C2= Untreated control plants of Desi variety, V2N= Plants sprayed with 5ppm Ag-nanoparticles, V1P1= Seeds treated with *Pseudomonas putida*, V1P2= Seeds treated with *Pseudomonas stutzeri*, V1NP1= Seeds treated with *Pseudomonas putida* and plants sprayed with 5ppm Ag-nanoparticles, V1NP2= Seeds treated with *Pseudomonas stutzeri* and plants sprayed with 5ppm silver-nanoparticles.

**Fig. (2).** Effects of PGPR and Ag-nanoparticles on the proline content of leaves.

In V2, the higher flavonoids content was recorded in all the treatments over control V2C2. The maximum flavonoids content was 62% in V2N (plants sprayed with Ag-nanoparticles) as compared to control V2C2.

### 3.11. Chlorophyll Content

#### 3.11.1. Chlorophyll a Content

Fig. (5) revealed that in V1, chlorophyll a was increased in all the treatments. The maximum increase was 31% in V1P1 (treatment with *Pseudomonas putida*) as compared to control V1C1.

In V2, the content of chlorophyll a was decreased in V2P1 and V2P2 plants. Treatment with Ag-nanoparticles alone or in combination with *Pseudomonas stutzeri* had either no significant effect or non-significant increase in chl a as compared to control.
3.11.2. Chlorophyll b Content

Fig. (5) showed that in V1 plants, the content of chlorophyll b was increased in all the treatments except in V1NP1 plants. The maximum chlorophyll b content was 33% in V1P2 plants (seeds treated with *Pseudomonas stutzeri*).

In V2, the stimulatory effects of the content of treatments were also observed. The maximum increase in the chlorophyll b was 23% in V2NP1 (seeds treated with *Pseudomonas putida* and plants sprayed with 5ppm Ag-nanoparticles) as compared to V2C2 plants.

3.11.3. Total Chlorophyll Content

In both V1 and V2, the total chlorophyll content was increased in all the treatments as compared to control V1C1 (Fig. 5). The maximum total chlorophyll content was 35% in V1NP2 (combined treatment with *Pseudomonas stutzeri* and Ag-nanoparticles) as compared to control. A similar response was observed in V2. The maximum content of total chlorophyll was 37% in V2NP1 plants (seeds treated with *Pseudomonas putida* and plants sprayed with 5ppm Ag-nanoparticles) than that of V2C2.

3.12. Carotenoids Content

In both V1 and V2, the content of carotenoids was increased in all the treatments as compared to control V1C1 (Fig. 6). The maximum increase in carotenoids was 35.4% in V1N plants (treated with Ag-nanoparticles) as compared to control. The maximum increase in carotenoids was 37% in V2N (plants sprayed with Ag-nanoparticles) as compared to control.

3.13. Sugar Content

Fig. (7) revealed that the content of sugar was increased in all the treatments in V1 and V2. The maximum increase in sugar content was 22% in V1NP1 (combined treatment with
3.14. Phenylalanine Ammonia-lyase Activity

In V1 as well as in V2, the PAL activity was increased in all the treatments of both the varieties (except in V1P1) as compared to control V1C1 (Fig. 8). The maximum increase of PAL activity was 29% in V1NP2 (combined treatments with *Pseudomonas stutzeri* and Ag-nanoparticles) as compared to control V1C1. The maximum PAL activity was 35% in V2N (plants sprayed with Ag-nanoparticles) as compared to V2C2.

3.15. Superoxide Dismutase (SOD) Activity

The SOD activity of leaves was increased in all the treatments as compared to control in both the varieties Fig. (9). Ag-nanoparticles alone and in combination with *Pseudomonas putida* enhanced the SOD activity in both the varieties. The SOD activity was greater in Poinsett 76 than that of Sialkot selection.
In V1, the maximum SOD activity was (75%) in V1N (plants sprayed with Ag-nanoparticles) than that of V1C1. In V2, the SOD activity was maximum (95%) in V2NP2 (plants combined treated with *Pseudomonas putida* and Ag-nanoparticles) as compared to V2C2.

### 3.16. Catalase (CAT) Activity

Catalase activity of leaves was increased in all the treatments as compared to control in both the varieties (Fig. 10). Ag-nanoparticles increased the catalase activity but
**Pseudomonas putida** and **Pseudomonas stutzeri** in combination with Ag-nanoparticles further augmented the catalase activity in both the varieties.

In V1, the maximum catalase activity was 50% in V1NP2 (combined treatments with **Pseudomonas stutzeri** and Ag-nanoparticles) than that of control V1C1. In V2, the maximum catalase activity was 31% in V2NP1 (plants treated with Ag-nanoparticles and **Pseudomonas putida**) as compared to V2C2.

### 4. DISCUSSION

The bacterial genera are vital components of soils that maintain various biotic activities of the soil ecosystem to make it suitable for the nutrient uptake and sustainable crop production [27].

The growth vigor of the Poinsett 76 was much greater than that of the Sialkot selection. It is evident from the result that the Ag-nanoparticles (AgNPs) effectively enhanced the number of branches/plants but decreased the effectiveness of PGPR for the production of branches. More flowers were produced in response to **Pseudomonas stutzeri** inoculation in Poinsett 76. Desi variety (Sialkot selection) had produced less number of flowers. PGPR strain, **Pseudomonas** fluorescence was reported to enhance the plant height and number of flowers of tomato plants [28].

In both the varieties, **Pseudomonas putida** effectively increased the shoot length at maturity phase but Ag-nanoparticles decreased the length of shoot. A previous report [29] indicated the plant height and productivity was increased in different crops inoculated with **Pseudomonas**, **Azotobacter** and **Azospirillum** strains.

Result demonstrated that the effect of Ag-nanoparticles and PGPRs varied with the variety of cucumber. **Pseudomonas putida** enhanced the shoot weight in Poinsett 76 whereas; it was inhibitory in Sialkot selection. **Pseudomonas putida** appears to cope with adverse environmental conditions as it maintains the turgidity of inoculated plants. PGPR inoculation improved the rate of seed germination, root development and root and shoot weight [30].

Roots were much longer in Desi variety (Sialkot selection) and Ag-nanoparticles further augmented it. In American variety (Poinsett 76), the Ag-nanoparticles inhibited the stimulatory effect of **Pseudomonas putida** (P1) and **Pseudomonas stutzeri** (P2). The effects of AgNPs on root growth showed both positive and negative effects depending on the studied plant species [31]. PGPR enhanced the growth of the plant and also improved the seed germination and root proliferation [32].

Fresh weight of root was much higher in Poinsett 76 and Ag-nanoparticles further augmented it. **Pseudomonas stutzeri** enhanced the fresh weight of root more effectively in Poinsett 76. The application of Ag-nanoparticles was inhibitory to both the PGPR for growth parameters but the inhibitory action was more pronounced with **Pseudomonas stutzeri**. PGPR inoculated plants receiving Ag-nanoparticle treatment had no significant effect on root weight [33]. Plants inoculated with PGPR (Plant growth-promoting rhizobacteria) increase the weight of root and shoot and the yield of many bowls of cereal crops [34].

The Ag-nanoparticles use the strategy to reduce the shoot fresh weight at the expense of an increase in root length and weight. This pattern of response was more evident in Desi variety (Sialkot selection). Plants with longer roots can adapt to moisture deficient soil as it can absorb water and nutrient deeper down the soil. Silver nanoparticles enhanced the fresh weight and length of root and shoot of **Brassica juncea** [35].

Proline accumulation plays a vital role in plant stress tolerance. The proline function as molecular chaperon that stabilize the structure of a protein and it may be a part of the
stress signal to initiate the adaptive response [36]. Proline accumulation within the cells acts as a signal to alleviate the salinity stress in plants [37]. Result demonstrated increased accumulation of protein following Ag-nanoparticles and PGPR (Pseudomonas putida and Pseudomonas stutzeri). In Sialkot selection, PGPR and Ag-nanoparticles were more effective for osmoregulant (proline) production under stress. Increase in proline production was reported in PGPR inoculated plants [38].

The response of the PGPR to chlorophyll and carotenoids content in plants varied. As observed that Pseudomonas putida enhanced the chlorophyll a in Poinsett 76 whereas; Pseudomonas stutzeri enhanced the chlorophyll b content in Poinsett 76. The combined effect of Pseudomonas putida and Ag-nanoparticles was more effective in Sialkot selection. Carotenoids are essential to protect the photosynthetic pigment system [39]. Ag-nanoparticles effectively enhanced the content of carotenoids in both the varieties as compared to control. PGPR strains stimulated the carotenoids content in both the varieties but in combination with Ag-nanoparticles, decreased the carotenoids content. The stimulatory effects of Ag-nanoparticles (chlorophyll a and b, total chlorophyll and carotenoids) were recorded in common bean (Phaseolus vulgaris) and corn (Zea mays) [40].

Phenols and flavonoids are antioxidants and free radical scavengers which also prevent the damage of cell due to oxidation and exhibit strong anti-cancer activity [41]. The apparently higher accumulation of phenolics content in leaves of cucumber induces the Sialkot selection to adapt to the stresses in a better way and also indicative of its greater potential for better human health. Ag-nanoparticles, Pseudomonas putida and Pseudomonas stutzeri significantly increased the phenolics content in both the varieties. Sialkot selection was more responsive to Ag-nanoparticles and PGPR treatments. Ag-nanoparticles increased the total phenolic content in leaves of plants [42]. Flavonoids are bioactive compounds found in fruits and vegetables. Higher intake of flavonoid-rich food has reduced the mortality from specific vascular diseases and cancers [43]. Flavonoids play a vital role to protect plants from different biotic and abiotic stresses and act as unique UV-filter and antimicrobial defensive compounds [44]. Sialkot selection was also more responsive to Ag-nanoparticles and PGPR treatments for flavonoids content. In plants, flavonoids are responsible to provide the color and fragrance to flowers and fruit to attract pollinators, also help in seed germination, growth and development of seedling. Total phenolic compound and total flavonoids were higher in Ag-nanoparticles treated plant extracts [45].

The sugar content in leaves was higher in Sialkot selection as compared to Poinsett 76. Pseudomonas putida in combination with Ag-nanoparticles further enhanced the sugar content significantly over control. Increase in sugar and phenolic content were recorded in plants treated with PGPR [46].

Higher protein content was found in leaves of American variety (Poinsett 76) and Pseudomonas putida further augmented it. Ag-nanoparticles effectively enhanced the leaf protein production in both the varieties. Inoculation of plants with native suitable PGPR may increase soluble protein content [47]. Ag-nanoparticles enhanced the protein content and carbohydrates in tested crop plants [48].

Phenylalanine ammonia-lyase (PAL) was the first enzyme of phenylpropanoid metabolism that plays an important role in the regulation of biosynthesis of phenols in plants [49]. PAL is an inducible enzyme that responds to biotic stresses such as pathogens and involved in plant defence. PAL has been generally recognized as a marker of environmental stresses, in different plant species [50]. Phenylalanine ammonia-lyase (PAL) activity was higher in Poinsett 76. In Poinsett 76, Pseudomonas putida and Pseudomonas stutzeri in combination with Ag-nanoparticles enhanced the PAL activity. In Sialkot selection, PAL activity was maximum in Ag-nanoparticles treated plants. PGPR strains induce systemic resistance in plants through activation of different defense-related enzymes, such as phenylalanine ammonia-lyase (PAL) [51]. PGPR strains enhance the enzyme activities of PAL [52].

Antioxidant enzymes such as Superoxide Dismutase (SOD), Peroxidase (POX), and Catalase (CAT) protect plants against the oxidative stresses [53]. Within a plant cell, Superoxide Dismutase (SODs) protect the plants against oxidative stress by detoxification of Reactive Oxygen Species (ROS) [54]. Results demonstrated that all the treatments stimulated SOD activity whereas Ag-nanoparticles alone and in combination with Pseudomonas putida were more effective in both the varieties. The SOD activity was increased in the runner bean plants inoculated with PGPR [55]. Catalase (CAT) plays a major role to provide protection against oxidative stress. Ag-nanoparticles enhanced the Catalase (CAT) activity in leaves of plants, thereby reducing the ROS and decreasing the toxicity in plants [56]. Catalase activity was less in untreated plants of Sialkot selection. The response of Ag-nanoparticles alone and in combination with Pseudomonas putida was more pronounced in Sialkot selection. Ag-nanoparticles enhanced the activity of antioxidant enzymes such as SOD and catalase in castor, bean seedlings [57].

**CONCLUSION**

The effect of PGPR and Ag-nanoparticles varied with the developmental stage and plant variety. Ag-nanoparticles can be implicated to promote length of the root; the response was different in both the varieties. Plants with longer roots can adapt to moisture deficient soil as it can absorb water and nutrient deeper down the soil. Ag-nanoparticles enhance the activities of defence-related enzymes. Ag-nanoparticles inhibit the efficiency of plant growth-promoting rhizobacteria (PGPR) and hence cannot be used in combination. PGPR use both sugar and proline to enhance osmoregulation. Pseudomonas putida was more effective as compared to Pseudomonas stutzeri in Sialkot selection.

**CURRENT AND FUTURE DEVELOPMENTS**

Pseudomonas putida may be used further either alone and in combination with Ag-nanoparticles to enhance the antioxidant and defence related enzyme activities. Ag-nanoparticles alone and in combination with PGPR can
effectively promote the detoxification of H$_2$O$_2$ generated under oxidative stresses.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**
Not applicable.

**HUMAN AND ANIMAL RIGHTS**
No animals/humans were used for studies that are base of this research.

**CONSENT FOR PUBLICATION**
Not applicable.

**AVAILABILITY OF DATA AND MATERIALS**
The authors confirm that the data and materials supporting the findings of this study are available within the article.

**FUNDING**
None.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest, financial or otherwise.

**ACKNOWLEDGEMENTS**
Declared none.

**REFERENCES**


