Pak. J. Bot., 52(4): 1473-1480, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-4(41)

NANOPARTICLES AND PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) MODULATE THE PHYSIOLOGY OF ONION PLANT UNDER SALT STRESS

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Abstract

The effects of rhizobacteria and Ag-nanoparticles were studied on the growth of onion seedlings under induced salt stress. The onion seedlings were treated with 50mM NaCl for 7days. The PGPR strains *Bacillus pumilus* and *Pseudomonas moraviensis* were used to inoculate one month old onion seedlings by adding broth culture in rhizosphere soil. One month after transplantation. Ag-nanoparticles (5ppm) were applied to rhizosphere soil for 5days. Number and weight of leaves, length of shoots, weight of roots, leaves and bulb were measured. 38 days are growth period after transplantation, the treated plants were analyzed for chlorophyll, carotenoids, sugar, protein, proline, flavonoids and phenolics content of onion leaves and bulb. *Bacillus pumilus* in association with Ag-nanoparticle performed better for growth stimulation of the onion plants. The soil moisture was higher in salt stressed plants but the PGPR inoculated plants and silver nanoparticles alone and also in combinations with PGPR exhibited decrease in the salt induced retention in soil moisture. Silver-nanoparticle combination with PGPR and alone increased the total chlorophyll and carotenoids contents under salt stress. Both Ag-nanoparticle and PGPR exhibited maximum increase in protein content of bulb, decreased the leaf flavonoids but had significant increase in the bulb flavonoid contents. The PGPR being more effective. The Ag-nanoparticle significantly increased the sugar and proline contents. *Bacillus pumilus* proved to be more effective under unstressed conditions to all growth parameters but *Pseudomonas moraviensis* effectively coped under salinated conditions. PGPR strains overcame the salt induced inhibition in growth parameters of plans. New proteins appear to be synthesized both by PGPR as well as Ag-nanoparticles to combat adverse effects of salt on plant growth.

Key words: Onion, PGPR, Ag-nanoparticles, NaCl, *Bacillus pumilus*, *Pseudomonas moravien*.

Introduction

Allium cepa L. (Onion) is the major crop in tropical countries comparable to other vegetables and crop plants and used daily in almost every meal. The common name of onion is Kanda, Pyaz and onion is an annual bulbous herb. The active ingredient is *allicin*, a sulfuric compound that's abundant across the *Allium* genus. Onions have phenols and flavonoids - especially, quercetin, sulfur, ascorbic acid (vitamins C), pyridoxine (B_6), and folic acid (B_9). Vit. B and vit. C, potassium and minerals Ca and Fe were also present.

Environmental constraints pose threat to crop productivity and perturb the physiology of plants. Plant morphology deleteriously effected by salt stress, osmotic and ionic stress also affects the physiology of plants, through these stresses biochemical responses changes occur in plants. (Khan *et al.*, 2013). Khidr *et al.*, (2010) treated wheat seedlings growing under natural conditions to NaCl 15, 30 and 45 mM. It was found that the concentration of soluble protein is decreased. According to El-Raslan (2007) salinity induced oxidative stress and proline accumulates in lettuce plant and proline reduce the NaCl-induced oxidative stress.Khalafallah *et al*., (2008) studied the effect of salts stress on faba bean. They found that salinity commonly reduced chlorophyll content but increased carotenoids content.

Salt stress is the most devastating stress for plant growth and productivity particularly in arid regions. According to Khan & Duke (2001) the world scenario for cultivated land revealed that 37% is sodic and 23% is saline. The main effects of salinity and sodicity are osmotic imbalance and ion toxicity (Tester & Devenport, 2003), that result in nutrient deficiency and nutrient imbalance (El-Wahab, 2006).

Plant growth promoting rhizobacteria (PGPR) form close association with the root of higher plants. (Wu *et al.*, 2005). The effects of PGPR vary with the species of plant, age, phonological stages and edaphic factors (Werner, 2000). The rhizobacteria inoculation regulates osmoregulant production e.g proline and also overcomes oxidative stress induced under salinity as dehydration stress (Bharti *et al*., 2016). Inoculated plants have increased nutrient uptake, greater leaf area for photoassimilation and higher chlorophyll and protein content (Dobbelaere, 2003).

The phenols which play important antioxidants are also stimulated by the PGPR inoculant (Barka *et al*., 2006). Among the osmoprotectants are sugars, alcohol (myoinositol), quaternary ammonium compounds such as proline, glycine, betaine and choline are worth mentioning (Bohnert & Jensen, 1996; Yokoi *et al*., 2002). The rhizobacteria are also involved in the enhanced production of these compounds. Shukla *et al*., (2012) reported that growth parameters (plant height, shoot length, root length, shoot dry weight root dry weight, and total biomass) were significantly higher in inoculated plants compared to control.

Only a few studies on silver nanoparticles (AgNPs) were done on plants (Krishnaraj *et al*., 2012; Monica & Cremonini, 2009). Plant growth and development effected by nanoparticles both positively and negatively. AgNPs (silver nanoparticles) have recently emerged a good candidate for research because of their positive effect on plant growth and yield at low concentration. Their role in the antioxidant enzyme production e.g catalase and peroxidase has also been demonstrated (Krishnaraj *et al*., 2012). The antimicrobial property of Ag-nanoparticle

depends on the species of bacteria (Shoults-Wilson *et al.*, 2011). Biochemical attributes (chlorophyll, carbohydrate and protein contents, antioxidant enzymes) of plants enhanced by silver nanoparticles (Salama, 2012; Sharma *et al*., 2012).

Present investigation was aimed to study the effect of PGPR and silver nanoparticles on growth and metabolism of onion seedlings exposed to NaCl stress and also evaluate the effect of Ag-nanoparticles alone and in combination with PGPRs (*Bacillus pumilus* and *Pseudomonas moraviensis*) on physiology and biochemical parameters of onion seedlings.

Materials and Method

Preparation of inocula: Single isolated colonies of bacteria (*Bacillus pumilus* and *Pseudomonas moraviensis*) was used to inoculate LB media and for 10 minutes inocula was centrifuged at 3000 rpm and the supernatant OD was measured at 660nm. Seedlings of onion one month old were transplanted in pots. Pots were filled with soil and sand mixture (3:1) and 1ml of inocula was given to each seedling, having two seedlings per pot. After 30 days of transplantation of the onion seedlings, 14 pots were treated with a solution of 50mM NaCl for 7d. Another group of 14 pots were treated with (5ppm) silver nanoparticles for 5 d. 5 pots remain untreated and served as control.

Soil moisture content: The soil moisture content was measured by taking 5g fresh weight of soil sample collected from 6 inches depth. The dry weight of soil was calculated by oven drying samples for 72 h at 70°C soil moisture content was calculated by the formula:

$$SMC\ (\%) = FW\text{-}DW \times 100/DW$$

Roots weight: Freshly harvested onion roots were weighed.

Chlorophyll & carotenoids: Chlorophyll and carotenoids estimations were made according to Arnon (1949). Fresh leaves (0.1g) were mixed with 5ml of 80% (w/v) acetone. Homogenized mixture was centrifuged at 2000 rpm for 5mins to clear the suspension. The supernatant was used for chlorophyll determination. The OD of the solution was measured at 645nm (chlorophyll a), 663nm (chlorophyll b) and 480 nm (carotenoids). Acetone (80%) was used as blank.

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 (100mg plant tissue) = 4 × OD × Total sample vol. /wt. of fresh plant tissue

Sugar content: The leaves sugar content was measured by the method of Dubois *et al.*, (1956) as modified by Johnson *et al*., (1996). Take 0.5g of plant tissue and homogenized in 10ml distilled water, then for 5 min centrifuged at 3000 rpm. 1ml of 5% phenol was add in 30ul of collected supernatant. Then incubate at room temperature for 1 hour. The concentrated sulphuric acid (5ml) was added. The absorbance (O.D) of each sample was recorded at 420 nm.

Protein content: The method of Lowry *et al*., (1951) was followed for protein determination in leaves. Bovine Serum Albumin (BSA) was used as standard for quantification of protein content of leaves.

Proline content: The leaves and the bulb proline content were determined following the method of Bates *et al.*, (1973). 0.5g of plant tissue was grinded in 5ml of 3% aqueous sulphosalicylic acid. Filtrate (2ml) was taken in a test tube to which were added glacial acetic acid (2ml) and acidic ninhydrin reagent (2ml) and after heating at 100°C for 1h.Then cooling at room temperature. The toluene (4ml) was added to the reaction mixture and the color intensity of the toluene was measured at 520nm against toluene blank. The amount of proline was calculated from the following formula:

Proline content (mg. g-1) = K value × dilution factor × Absorbance (O.D)/weight of the sample
K value = 19.6

Flavonoids content: The flavonoid content was determined following the method of Zhishen *et al.*, (1999). The leaves homogenate prepared in 80% methanol and were allowed to centrifuge for 10 min at 3000 rpm. $AlCl_3$ reagent was prepared by taking 133mg crystalline $AlCl_3$ and 400mg crystalline sodium acetate, dissolved in 100ml of 80% methanol. Water (400µl) and 1ml of $AlCl_3$ reagent were added to 2ml of supernatant. After thorough mixing the absorbance was recorded at 430nm against blank.

Phenolics content: Gallic acid as standard Method of (Singleton & Jones, 1999) was used to determine the phenolic content of leaves. Gallic acid was used as standard.

Results

The rhizosphere soil moisture content: The soil moisture content was (113%) higher in rhizosphere soil of plants treated with NaCl as compared to untreated control. The rhizosphere soil of plants inoculated with *Bacillus pumilus* and *Pseudomonas moraviensis* (T3 & T4) and treated with Ag-nanoparticles had no significant difference with the control (Table 1) though *B. pumilus* inoculated plants rhizosphere soil had slightly higher soil moisture retained in the soil. Under salt stress the 2 PGPR were equally effective and retained the moisture in the rhizosphere soil lower than that of salt treatment made alone. Furthermore, the combined treatment of Ag-nanoparticles and PGPR showed soil moisture content lower than the salt treatment made alone.

Table 1. Fresh weight of root, bulb, green leaves and senescent leaves and soil moisture content.

Treatments	Soil moisture content (%)	Fresh root weight (g)	Fresh weight of onion bulb (g)	Fresh weight of green leaves (g)	Weight of senescent leaves (g)
C1	6.15	0.38 ± 0.08	1.49 ± 0.43	1.38 ± 0.29	0.14 ± 0.38
C2	13.12	0.22 ± 0.02	1.58 ± 0.22	1.31 ± 0.36	0.16 ± 0.05
C3	5.93	0.35 ± 0.04	1.54 ± 0.42	1.29 ± 0.37	0.05 ± 0.0
T1	8.22	0.49 ± 0.38	2.61 ± 0.63	2.23 ± 0.69	0.11 ± 0.02
T2	6.6	0.34 ± 0.07	1.98 ± 0.45	1.63 ± 0.54	0.09 ± 0.03
T3	10.86	0.29 ± 0.03	1.34 ± 0.40	1.49 ± 0.38	0.15 ± 0.04
T4	10.37	0.30 ± 0.02	1.70 ± 0.22	1.31 ± 0.21	0.12 ± 0.02
T5	5.48	0.40 ± 0.10	1.92 ± 0.44	1.44 ± 0.35	0.04 ± 0.01
T6	7.06	0.39 ± 0.09	1.81 ± 0.49	1.60 ± 0.42	0.06 ± 0.02

Treatments detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis*, T3= Plant treated with *Bacillus pumilus* + salt,T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs),T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress

Effect on plant growth: Salt treatment decreased (42%) the fresh weight of roots. All the treated plants had shown increase in bulb, root and leaves weight as compared to untreated control. The plant treated with PGPR, *Bacillus pumilus* and *Pseudomonas moraviensis* in combination with silver nanoparticle (AgNPs) had shown decrease in weight of senescent leaves as compared to untreated control (Table 1).

The onion fresh weight of bulb was slightly increased in both the salt treatment and Ag-nanoparticle treatment. *Bacillus pumilus* and *Pseudomonas moraviensis* inoculation significantly increased the fresh weight of onion bulb by 75 % and 33% respectively. PGPR alone and in combination with silver-nano particles enhanced the fresh weight of senescent leaves (Table 1).

The method of Lowry *et al*., (1951) was followed for protein determination in leaves. Bovine Serum Albumin (BSA) was used as standard for quantification of protein content of leaves.

Chlorophyll content: The chlorophyll .b content and total chlorophyll content were significantly higher in salt stressed and Ag-nanoparticle treatments. The latter being more stimulatory. Although PGPR inoculation made alone and in combination with silver-nano particles augmented the Chl.b and total Chl. content of plant but the PGPR effect was more pronounced under salt stress (Fig. 1). Noteworthy, the *P.moraviensis* was more stimulatory. Both the PGPR significantly stimulated carotenoids content of leaves. PGPR effectively alleviated the salt and Ag-nano particle induced decrease in the carotenoids content of onion leaves (Fig. 2).

Sugar content: Salt treatment reduced the leaves sugar content in bulb accumulation of sugar was observed. On the contrary the Ag-nanoparticle significantly increased the leaf sugar content having no significant effect on bulb sugar content. The plants treated with *Bacillus pumilus* and *Pseudomonas moraviensis* in combination with silver nanoparticle (AgNPs) and 50 mM NaCl had shown increase in bulb and leaves sugar content as compared to plants treated with PGPRs alone. *B. pumilus* had stimulated bulb sugar content while reducing the leaf sugar content and also ameliorated the salt induced inhibition in the leaf sugar content (Fig. 4).

Protein content: Both the PGPR alone and in association with Ag-nanoparticles enhanced the protein content of the bulb significantly over C. The ameliorative effect of PGPR was also evident under salt stress (Fig. 5).

Proline production: The proline production of bulb was increased following Ag-nano particles treatment but reduction in the leaf proline was observed. Whereas, PGPR increased the leaf proline but *P.moraviensis* decreased proline content in bulb *or B. pumilus* has similar response was exhibited under salt stress (Fig. 3).

Flavonoids content: The flavonoids content of bulb was increased without affecting that of leaves under salt stress. *P. moraviensis* and *B. pumilus* inoculations under salinity resulted in significantly higher flavonoids accumulation was observed in leaves (Fig. 6). As compared to untreated control Ag-nanoparticle in combination with PGPR were decreased the leaf flavonoids but had significant increase in the bulb flavonoid content. However, as compared to Ag-nano particle applied alone the flavonoid content of leaves were significantly higher having no significant effect on the flavonoid content of bulb.

Phenolic content: Results presented in (Fig. 7) indicated significant accumulation of phenolics in leaves of PGPR treatments. The *P. moraviensis* being less effective than *B. pumilus* when applied alone and also under salt stress. Inoculation with *B. pumilus* was ineffective under salt stress or in the presence of silver-nanoparticles. Silver-nanoparticles alone were inhibitory to leaf phenolics content. The phenolic content of the bulb was significantly increased by PGPR also under salt stress the value was higher. *B. pumilus* was more effective but under salt stress *P. moraviensis* was increased the phenolic content in bulb significantly were untreated control (C1 and also salt stressed plants C2 and Ag-nanoparticle treated plants C3). Ag-nano particle has no significant effect (Fig. 8).

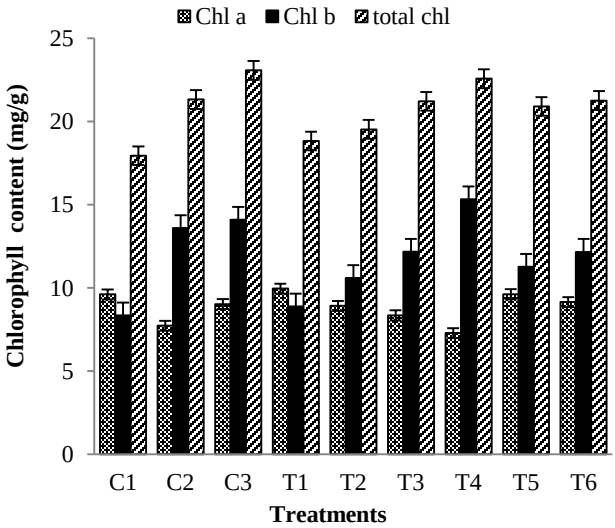

Fig. 1. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl and PGPRs alone on onion chlorophyll a, chlorophyll b and total chlorophyll content. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

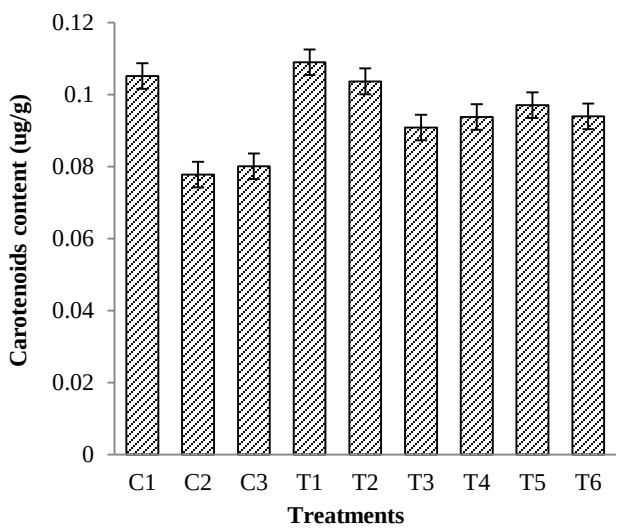

Fig. 2. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion carotenoids content. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

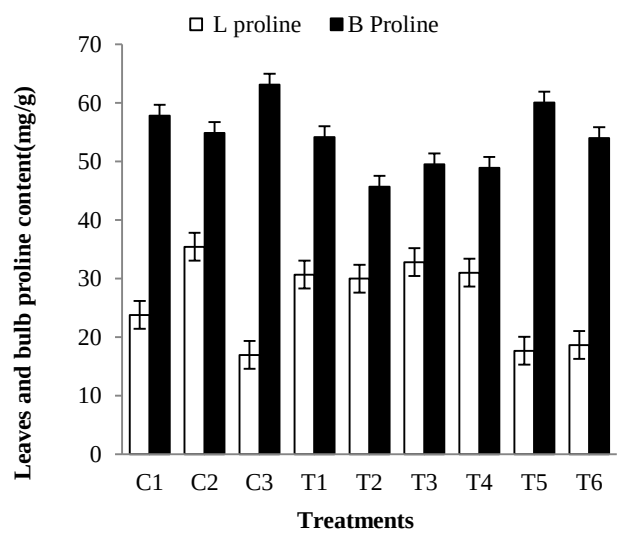

Fig. 3. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion proline content in leaves and bulb (L protein= Leaf protein, B protein= Bulb protein). Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

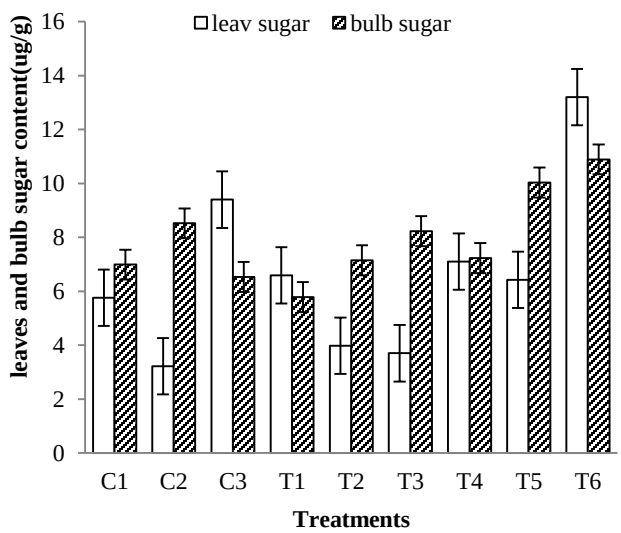

Fig. 4. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion sugar content in leaves and bulbs. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

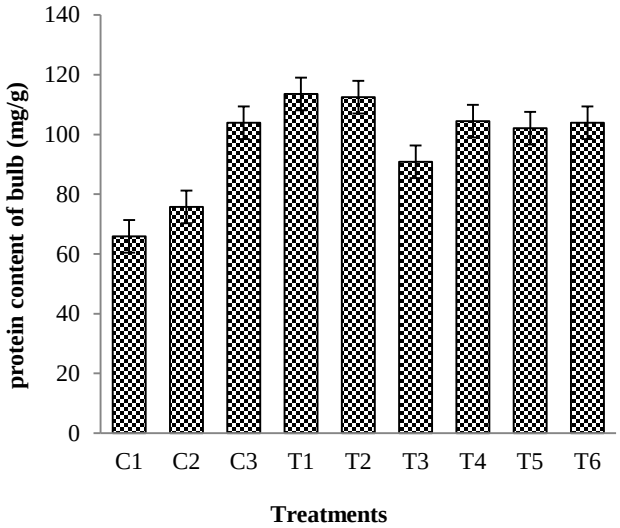

Fig. 5. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion protein content in bulbs. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

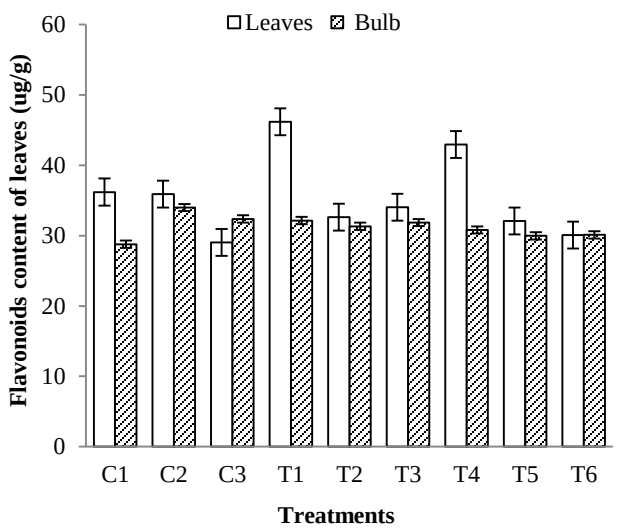

Fig. 6. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion flavonoids content in leaves and bulb. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

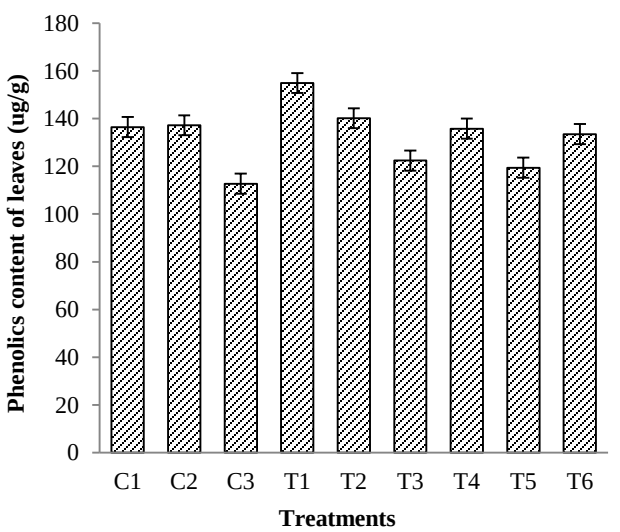

Fig. 7. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion phenolics content in leaves. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

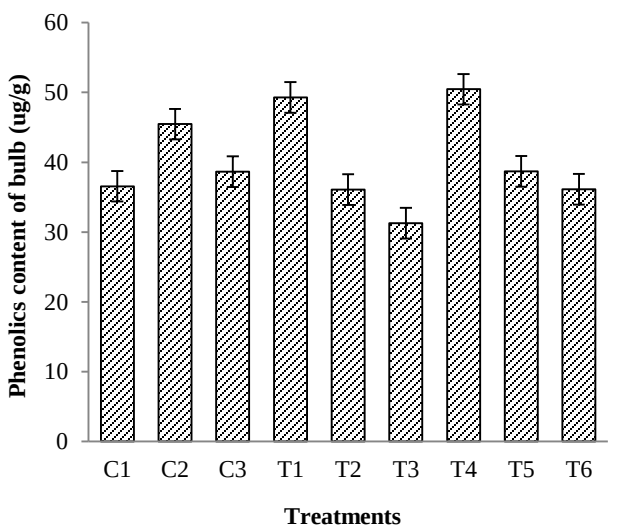

Fig. 8. Effect of PGPRs in combination with silver nanoparticles (5ppm) and 50mM salt (NaCl) and PGPRs alone on onion phenolics content in bulb. Treatments Detail: C1= Untreated control, C2= Plant treated with 50 mM salt (NaCl), C3= Plant treated with silver nanoparticle (AgNPs, 5ppm), T1= Plant treated with *Bacillus pumilus*, T2= Plants treated with *Pseudomonas moraviensis* , T3= Plant treated with *Bacillus pumilus* + salt, T4= Plants treated with *Pseudomonas moraviensis* + salt, T5= Plant treated with *Bacillus pumilus* + sprayed 5ppm silver nanoparticle (AgNPs), T6= Plant treated with *Pseudomonas moraviensis* + sprayed 5ppm silver nanoparticle (AgNPs). Salt stress was applied at 30 days after transplanting and treatments were made at 38 days after salt stress.

Discussion

The observed increase in soil moisture (113%) in the rhizosphere soil of salt stressed onion seedlings demonstrates salt induced inhibition in water and nutrients in the rhizosphere. Results further demonstrate that PGPR and Ag-nanoparticles overcame this inhibition *P. moraviensis* was more effective under saltness. The PGPR ameliorated the osmotic effect of salt stress and allowed the root for enhanced uptake of water and nutrients as evidenced by decrease in salt moisture(24 %) available in the rhizosphere of plants treated with PGPR + salt as compared to salt treatment made alone. The role of Ag-nanoparticle used alone as well as in combination with *Bacillus pumilus* was at par with control and indicated lower soil moisture % indicating better metabolic rate of water transport through roots. The interaction of Ag-nanoparticles with *Pseudomonas moraviensis* was also positive.

PGPR appears to assist Ag-nanoparticle to increase the fresh weight of onion bulb. The onion green leaves which are used in salads and as vegetables also constitute an important part of human diet. The significant effect of PGPR was also observed under unstressed condition on the fresh weight of green leaves of onion. Similar to that in bulb, green leaves also responded to PGPR which assisted Ag-nanoparticle under salt stress to enhance the fresh weight of green leaves. Both the PGPR alleviated the salt induced inhibition in root length but *Pseudomonas moraviensis* was have stimulatory. These bacteria were found to improve crop productivity in salt degraded land (Maheshwari *et al*., 2012;Yang *et al*., 2009).The plant growth in the presence of PGPR improved by the mechanism of biological nitrogen fixation, nutrient uptake biosynthesis of essential phytohormones, and by resistance to biotic and abiotic stresses (Kang *et al*., 2010; Richardson *et al*., 2009) The PGPR isolated from the local environment proves better in enhancing growth and defensive mechanism (Zhang *et al*., 2011; Ahmad *et al*., 2013). *Bacillus pumilus* and *Pseudomonas moraviensis* are involved in growth increase and better root proliferation and the bulb growth, but PGPR in the presence of Ag-nanoparticles affects the root growth negatively.

The stimulatory effects of PGPR were decreased by the Ag-nanoparticle for carotenoids content of onion leaves. The chlorophyll pigments (a, b and carotenoids contents) of maize was decreased, under salinity Nadeem *et al*., (2006). The chlorophyll content was reduced under abiotic stress conditions that is the main cause of chloroplast damages that are caused by active oxygen species (Manivannan *et al.*, 2007). According to El-Nimer *et al*., (1992) high salinity levels significantly decrease the leaves total chlorophyll content of Datura seedling. Also, El-khateeb (1994) showed that potted *Murraya exotica* L. seedling irrigated with saline water (1500 or 3000 ppm) resulted in decreases in Chlorophyll a, Chlorophyll b and carotenoids contents of leaves.

Under salt stress, the specific ion toxicities (e.g. Na+ and Cl–) and ionic imbalances acting on biophysical and/or metabolic components of plant growth and reduced plant growth (Grattan & Grieves, 1999). High salt concentrations affect the bacterial species by developing osmotic effects and specific ion effects that get disrupted in the rhizosphere zone. Plants and microbes combat with the osmotic and oxidative stress by accumulating osmolytes and the large amount of energy is required for synthesis of osmolytes resulting in significant decrease in growth and activity of plants (Wichern & Joergensen, 2006). The observed detection of higher proline production under salt stress in the leaves of onion seedling created due to salt. The tendency of Ag-nanoparticles to produce proline significantly lower than that of the untreated control both in the single application or combined application with PGPR suggest the ameliorated measure to overcome osmotic imbalance created under salt stress. The accumulation of amino acids (proline) in plants under salinity was recorded in many investigations (Moghaieb *et al*., 2004; El-Raslan, 2007). Under stress conditions many PGPR strains such as *Burkholderia* (Barka *et al*., 2006), *Arthrobacter*, and *Bacillus* (Sziderics *et al*., 2007), increase proline synthesis in plants, that helps the plant to cope with saltness and maintain the water status in cell. Proline also maintains the cell pH and enhances the activity of various enzymes, scavenging reactive oxygen species were helped to maintain antioxidant activity (Verbruggen & Hermans, 2008).

The increased flavonoids content were observed in the treatments with Ag-nanoparticles and PGPR. Salt stress is possibly attributed to the protective role of flavonoids against stress. Phenolic compounds are stress related compounds produced under adverse environmental conditions. *B. pumilus* was more effective but under salt stress *P. moraviensis* increased the phenolic content in the bulb. According to Lee *et al*., (2005) accumulation of isoflavone was observed in soybean plants that inoculated with various PGPR strains.

Noteworthy, are the augmentation in the bulb protein following the Ag-nanoparticle and PGPR application. Protein was increased in all the treatments inoculated with PGPR strains as compared to control. Inoculation of plants with native suitable PGPR may increase soluble protein content (Dobbelaere, 2003). It appears that in case of salt treatment the sugar translocation from leaves to bulb occurred whereas in Ag-nanoparticle treatment sugar content was increased in leaves but no translocation observed to onion bulb. The total soluble carbohydrates increased with increasing the concentration NaCl was observed in chaksu (*Cassia absus* L.) Hussain *et al*., (2009).

The sugar content, protein and the proline were augmented in the presence of PGPR and Ag-nanoparticles. The increase in sugar content of bulbs under salt stress may be due to the fact that the treated plants use sugar as osmoprotectant under salt stress. *Pseudomonas moraviensis* was more effective under salt stress. Ag-nanoparticle treatment exhibited maximum increase in sugar content of bulbs in combined treatment with PGPR. The observed decrease in leaf sugar under salt stress was alleviated by the PGPR, *Pseudomonas moraviensis* being more effective. The PGPR augmented the effect of Ag-nano particle.Results indicated that both the PGPR and Ag-nanoparticles augmented the protein content of onion bulb. Flavonoid content of both leaves

and bulb were not affected by Ag-nanoparticle alone but in presence of PGPR decrease in flavonoids content was recorded. The levels of aromatic amino acids such as cysteine, arginine, methionine and the protein content were decreased in plants under salinity. Proline accumulation within the cells acts as the signal to reduce the salt stress in plants (Matysik *et al*., 2002).

Conclusion

PGPR induce better water uptake and possibly nutrient from soil particularly under salt stress and increases onion bulb and weight of root effect was at par with Ag-nanoparticle. The silver-nanoparticle in combination with PGPR were more effective to increase onion bulb weight under saltness. The *Pseudomonas moraviensis* was more effective under salinity whereas *Bacillus pumilus* was promontory under unstressed condition. Ag-nanoparticles increases sugar content of bulb and leaves significantly higher than PGPR or control. *Pseudomonas moraviensis* was more effective to increase bulb phenolic content.

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(Received for publication 11 December 2018)