CHAPTER: 7

Comparative Analysis of Three Potent PGPR Strains in a Pot Experiment: Results with ANOVA Study

7.1 Pot experiment

The plants were treated with combined applications of PGPRs and ZnO NPs at 400 ppm concentration. The treatments were set up as follows: the control group consisted of plants that received no treatment. One group was treated with 400 ppm of ZnO NPs, while another group received zinc in the form of a salt. The experimental treatments included a series of combinations and individual applications aimed at understanding the synergistic and independent effects of Zinc Oxide nanoparticles (ZnO NPs) and specific bacterial strains. Combinations of 400 ppm ZnO NPs were combined with the bacterial strains *Pseudomonas songnenensis* (RG8), *Bacillus haynesii* (RG12), and *Priestia megaterium* (RGKP3), forming distinct treatment groups to evaluate their collective impact. Additionally, separate groups were treated exclusively with the bacterial strains *Pseudomonas songnenensis* (RG8), *Bacillus haynesii* (RG12), and *Priestia megaterium* (RGKP3) in the absence of ZnO NPs to assess the individual contributions of these microbes. This design enabled a comprehensive comparison between the effects of combined treatments and those of individual components.

7.1.1 Physical parameters

The physical characteristics of groundnut plants were extensively examined to evaluate the impact of combining ZnO NPs with PGPR. The results revealed that the combined application of PGPR with 400 ppm ZnO NPs significantly enhanced several key growth parameters, including the number of roots, leaves, branches and root length, when compared to untreated control plants. These findings highlight the beneficial effects of this combined treatment in promoting overall plant growth.

In contrast, plants treated with only zinc salt (400 ppm), only PGPR, or only ZnO NPs (400 ppm) exhibited comparatively lower growth performance, indicating that the individual treatments were less effective in stimulating plant growth. This suggests that the cumulative effect of PGPR and ZnO NPs plays a critical role in improving plant development, with each component contributing to enhanced nutrient uptake, root development, and overall plant health.

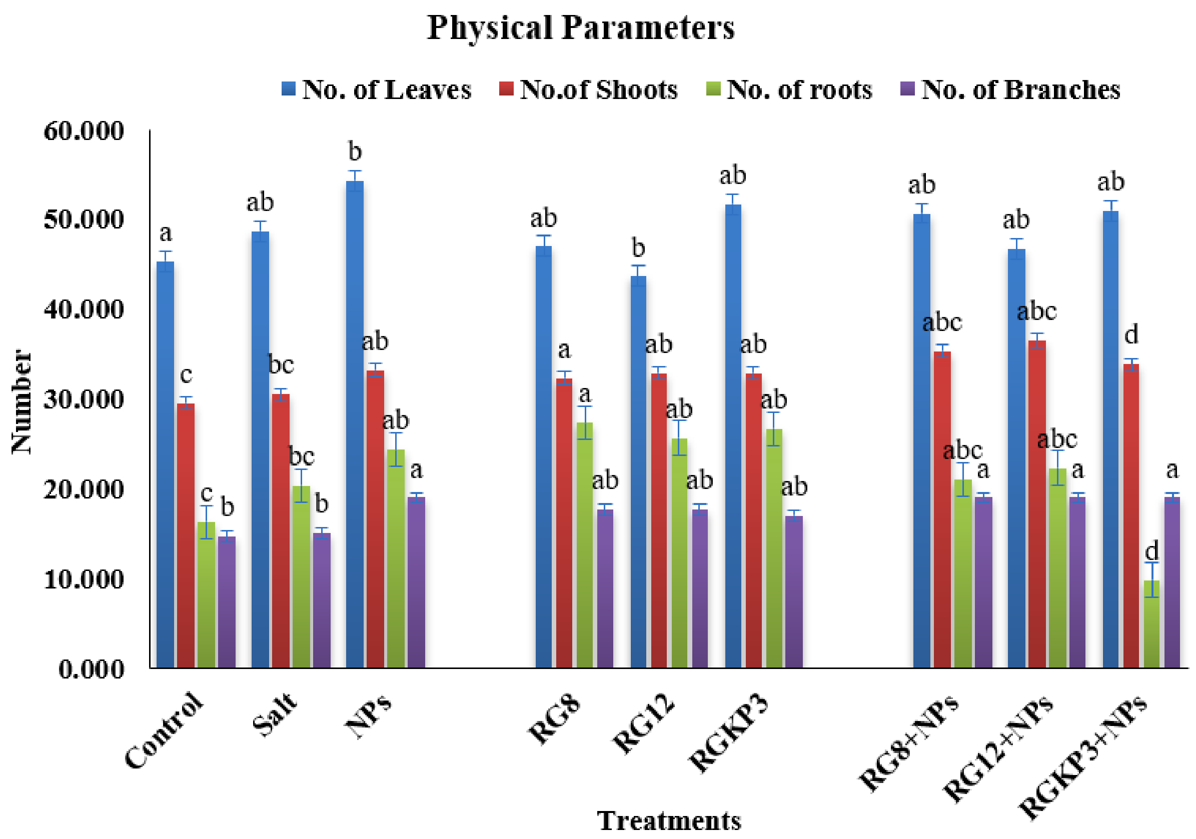
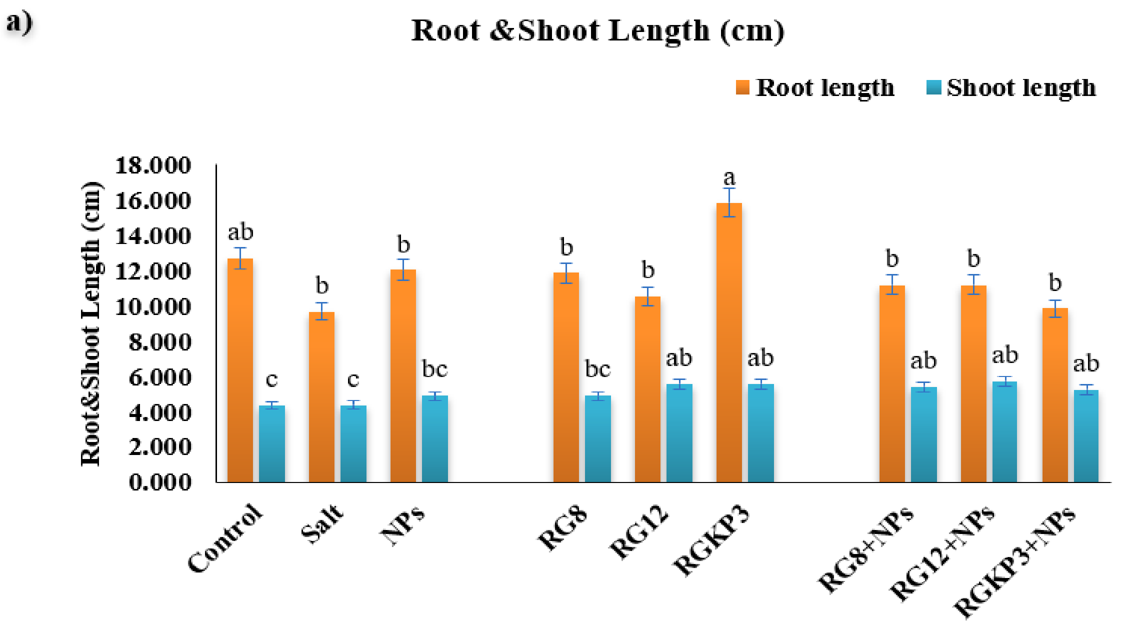


Figure 7.1: Plants with different treatments in pot experiment after 1 month; Physical parameters number of leaves, roots, shoots, and branches focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Among the various PGPR strains tested, *Priestia megaterium* (RGKP3) combined with ZnO NPs stood out as the most effective in improving growth characteristics. This combination produced the notable improvements in root number, leaf count, and root length compared to the other strains, *Bacillus haynesii* (RG12) and *Pseudomonas songnenensis* (RG8). The results suggest that *Priestia megaterium*, when used in combination with ZnO NPs, is particularly effective in promoting groundnut growth, likely due to its unique synergistic interactions with NPs. These findings offer valuable insights into the potential of combining PGPR and nanotechnology to optimize agricultural practices and improve crop productivity.



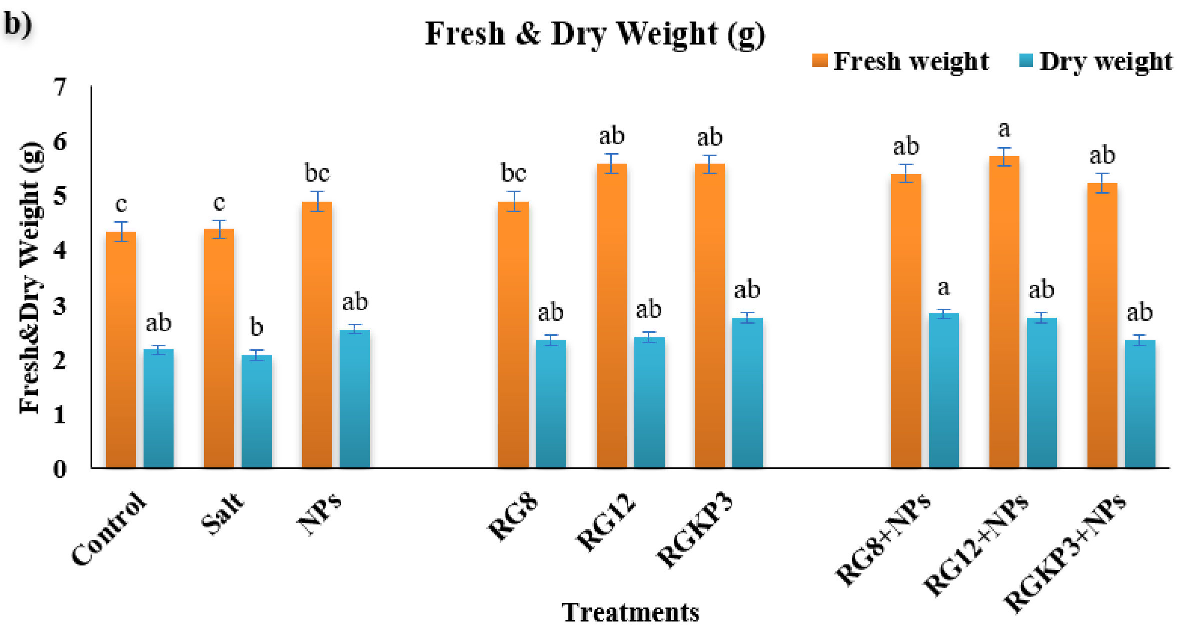


Figure 7.2: (a) Physical parameters such as root length and shoot length and (b) Physical parameters such as fresh weight and dry weight of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt and control (untreated) focusing on SSR expression.

Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

7.1.2 Biochemical parameters

The evaluation of biochemical parameters in treated plants demonstrated a significant enhancement in response to the combined application of PGPR and ZnO NPs. Notably, plants treated with this combination exhibited higher levels of biochemical compounds compared to those in the untreated control group or plants treated only with ZnO NPs. These findings indicate that the presence of PGPR not only complements the effects of ZnO NPs but also augments the plants overall physiological responses, resulting in improved biochemical performance.

7.1.2.1 Chlorophyll Content

Chlorophylls a and b play crucial functions in photosynthesis and are also effective singlet oxygen photosensitizers (Bashir et al., 2021). It was observed that chlorophyll a, b, and total chlorophyll were reduced in plants with only ZnO NPs treated, zinc salt treated, and untreated plants.

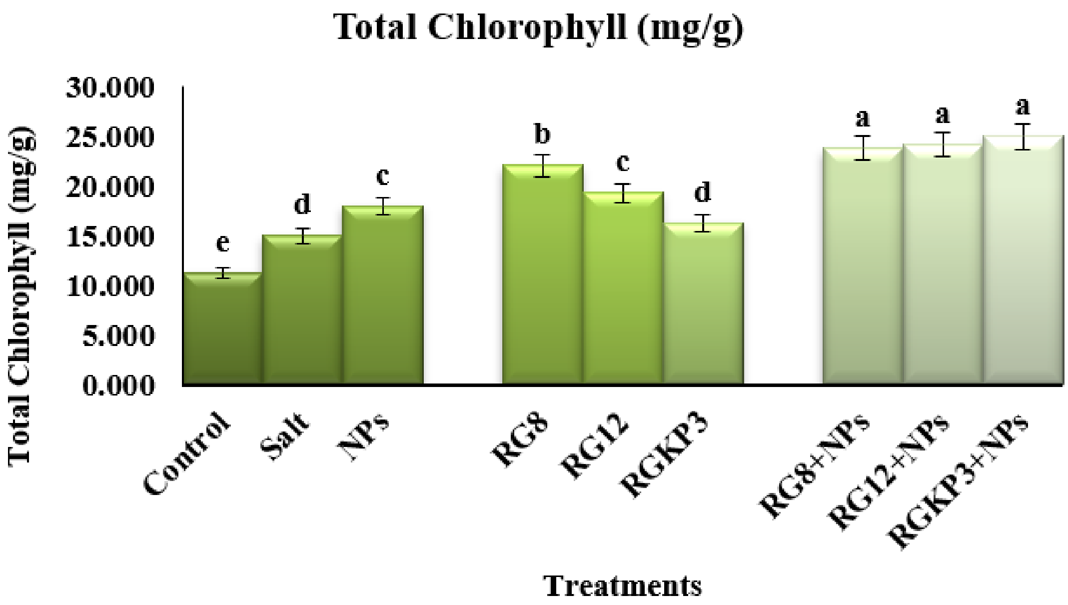


Figure 7.3: Total Chlorophyll Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

The application of PGPR combined with ZnO NPs significantly improved chlorophyll content in plants compared to those treated with only PGPR (RG8, RG12, and RGKP3). Among the strains, *Priestia megaterium* (RGKP3) with 400 ppm concentration of ZnO NPs demonstrated the highest total chlorophyll production, accomplishment 24.80 mg/g, outperforming the other two potent strains. It was also observed that all three potent strains with ZnO NPs were able to produce an almost two-fold value of produced chlorophyll as compared to untreated plants. Komal et al., (2022) observed that PGPRs (*Pseudomonas Aeruginosa*, *Pseudomonas Chlororaphis*, and *Fusarium oxysporum*) treatment positively influenced chlorophyll content in chickpea plants. Chlorophyll a and b concentrations showed notable variation at different time points after sowing (30, 60, and 90 days).

7.1.2.2 Carotenoid Content

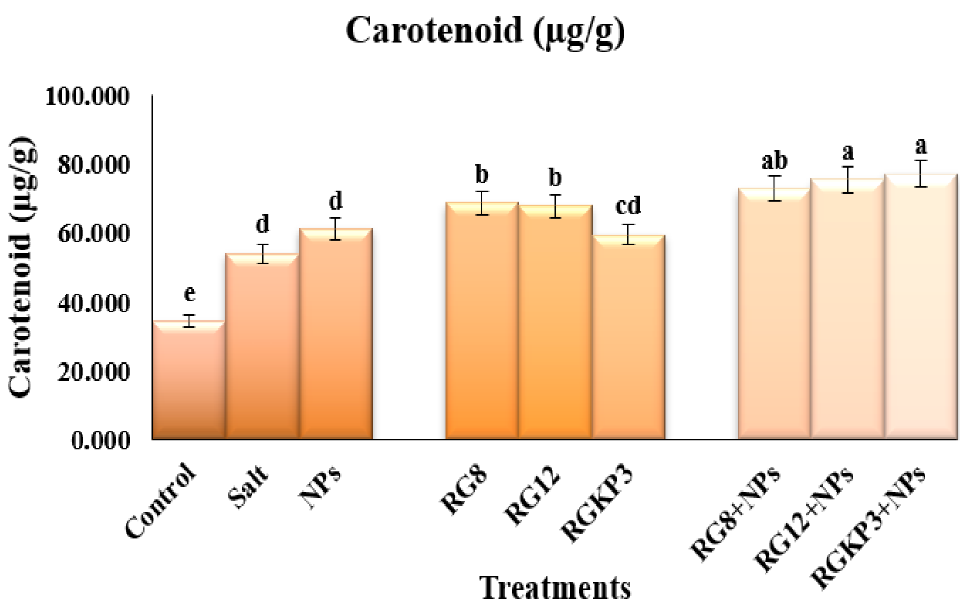


Figure 7.4: Carotenoid Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Carotenoid production in the leaves varied from 34.4 to 77.4 µg/g across treatments. Compared to untreated plants, plants treated with only zinc salt, or those treated with only

ZnO NPs, the combination of *Priestia megaterium* (RGKP3) and ZnO NPs demonstrated the highest carotenoid production (77.4 µg/g). Similarly, *Pseudomonas songnenensis* (RG8) alone produced 68.8 µg/g of carotenoids, while RG8 combined with 400 ppm ZnO NPs showed an increased production of 72.56 µg/g, outperforming other treated plants. Moreover, out of three potent *Priestia megaterium* (RGKP3) strains shows the maximum amount of carotenoid in the pot experiment. Plants treated only with NPs also had higher carotenoid levels than those treated with zinc salt or untreated.

7.1.2.3 Flavonoid Content

In addition, compared to other treated plants, the production of flavonoid was highest in plants treated with PGPR and NPs (RG8+ ZnO NPs, RG12+ZnO NPs, and RGKP3+ZnO NPs). Untreated plants produced minimum flavonoid compared to other treated plants. When comparing treated with untreated plants, the zinc oxide salt also produced a slightly higher amount (2.15 µg/g) of flavonoid.

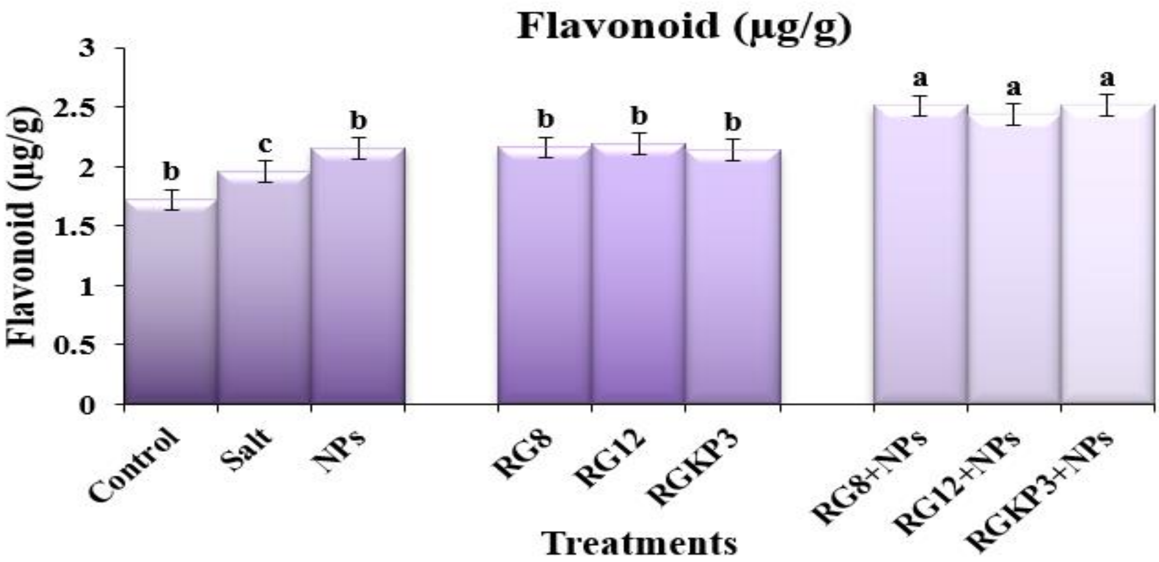


Figure 7.5: Flavonoid Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Three strains of PGPRs with ZnO NPs generate 2.44-2.51 µg/g of flavonoid which was 68.5% higher than untreated plants. Only PGPR-treated plants are also able to produce a high number of flavonoid compared to untreated and zinc salt-treated plants. *Priestia megaterium* (RGKP3) and *Pseudomonas songnenensis (RG8)* strains produced a similar amount (2.51 µg/g) of Flavonoid in the pot experiment as compared to *Bacillus haynesii* (RG12) strain (2.44 µg/g). Ham et al., (2022) reported that the PGPR strain *Pseudarthrobacter sp. NIBRBAC000502770* improved the growth and flavonoid content of *G. aleppicum*. The total flavonoid content in the methanol extract of *G. aleppicum* was found to be 35.5 mg/g.

7.1.2.4 Proline Content

The proline content of plant leaves shot up from 25.67 to 92.51 mg/g during the pot assessment. The treatment with RG12 and ZnO NPs produced the least proline content.

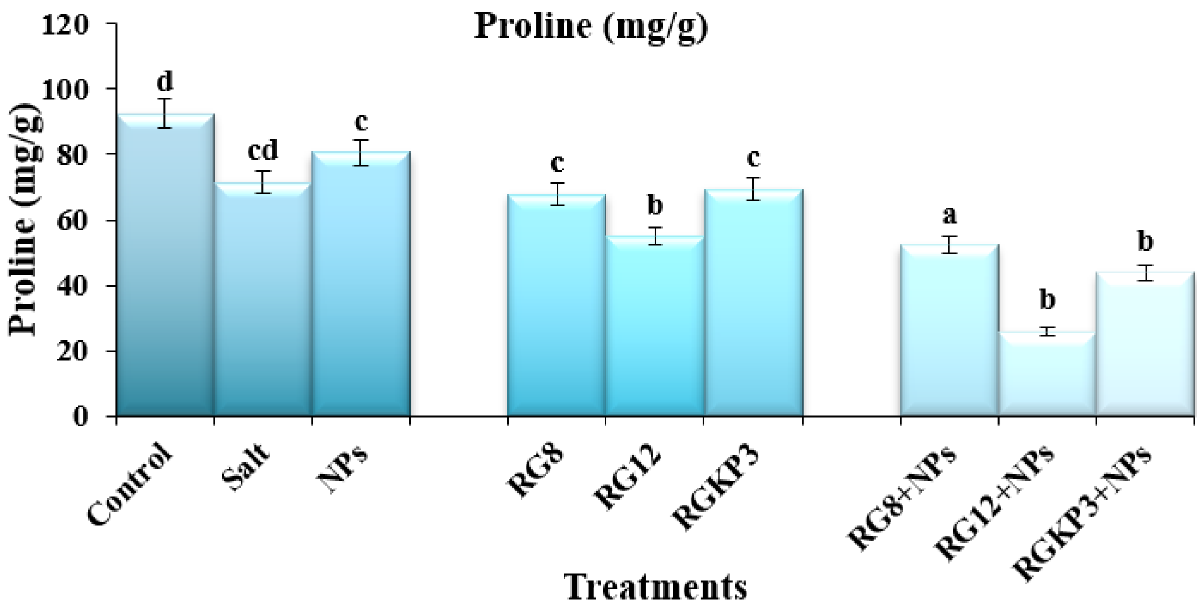


Figure 7.6: Proline Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figure 7.6 demonstrated that the proline produced in the range of 25.33-52.33mg/g in plants treated with PGPR and ZnO NPs. Untreated plants produced the maximum range of proline (92.51 mg/g), which was more than all treated plants. *Bacillus haynesii* (RG12) with ZnO

NPs produced the least amount of proline as compared to the other two strains *Priestia megaterium* (RGKP3) and *Pseudomonas songnenensis (RG8)*. Zinc salts produced the least amount of proline when it is compared to only NPs and only PGPRs. Zainab et al. (2021) reported *B. xiamenensis* decreased the proline content up to 117%, and *B. gibsonii* increased the proline content up to 112%.

7.1.2.5 Sugar Content

The highest total sugar content (134.19 mg/g) was observed in groundnut plants treated with the combined application of ZnO NPs and the PGPR strains RG8, RG12, and RGKP3, compared to untreated plants. In contrast, individual treatment with RG8, RG12, and RGKP3 resulted in similar sugar levels, ranging from 110.64 to 112.41 mg/g. Plants treated only with ZnO NPs also showed a significant increase in sugar content (132.09 mg/g) compared to both untreated plants and those treated only with PGPR strains. Among the combined treatments, RG8 (*Pseudomonas songnenensis*) with 400 ppm ZnO NPs produced the highest sugar content, as illustrated in Fig. 7.7. This aligns with earlier findings, such as those by Stefan et al. (2013), which demonstrated that PGPR strains could enhance the carbohydrate content of crops, improving their overall nutritional quality.

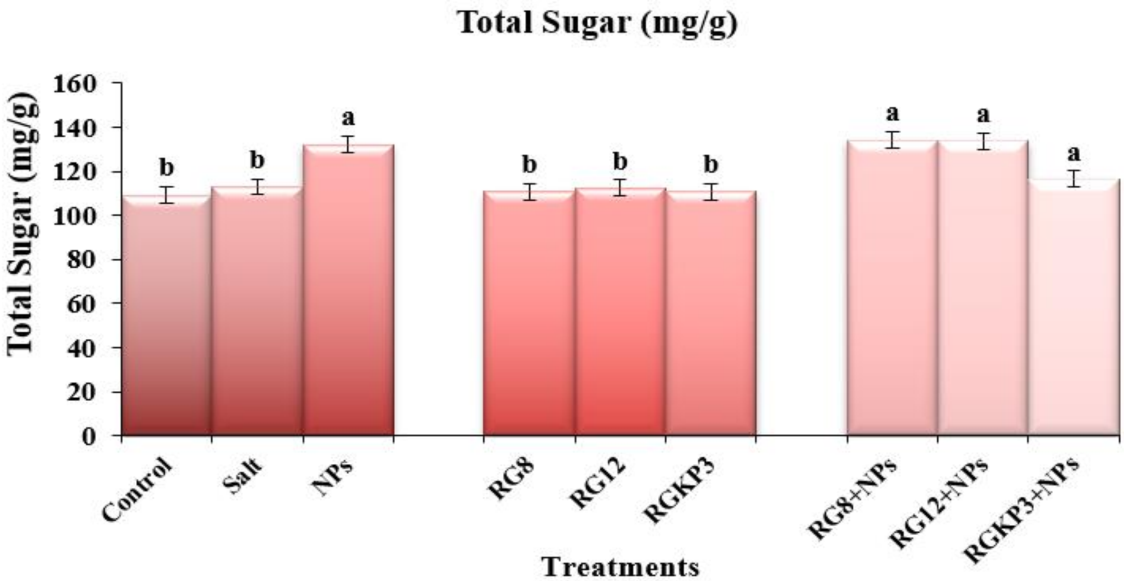


Figure 7.7: Total Sugar Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

7.1.2.6 Protein Content

As demonstrated in previous study, increased concentrations of ZnO NPs were associated with greater protein synthesis. Protein content was also high in all plants that were treated with the combination of PGPR and ZnO NPs. RG8 + NPs, RG12 + NPs, and RGKP3 + NPs treated plants produced higher amounts of total protein 134.19, 133.70, and 116.45 mg/g respectively as compared to other treated plants. RG8+NPs (*Pseudomonas songnenensis*) treated plants produced the highest protein as compared to the other two potent strains. When PGPR-containing NPs were compared with untreated plants, the amount of protein produced was about increased by twofold. Warwate et al. (2017) suggest that the combined application of three plant growth-promoting rhizobacteria (*Azotobacter, PSB, Pseudomonas*) and silicon fertilizer can improve the growth, yield, and biochemical activity of *P. vulgaris* in saline soils.

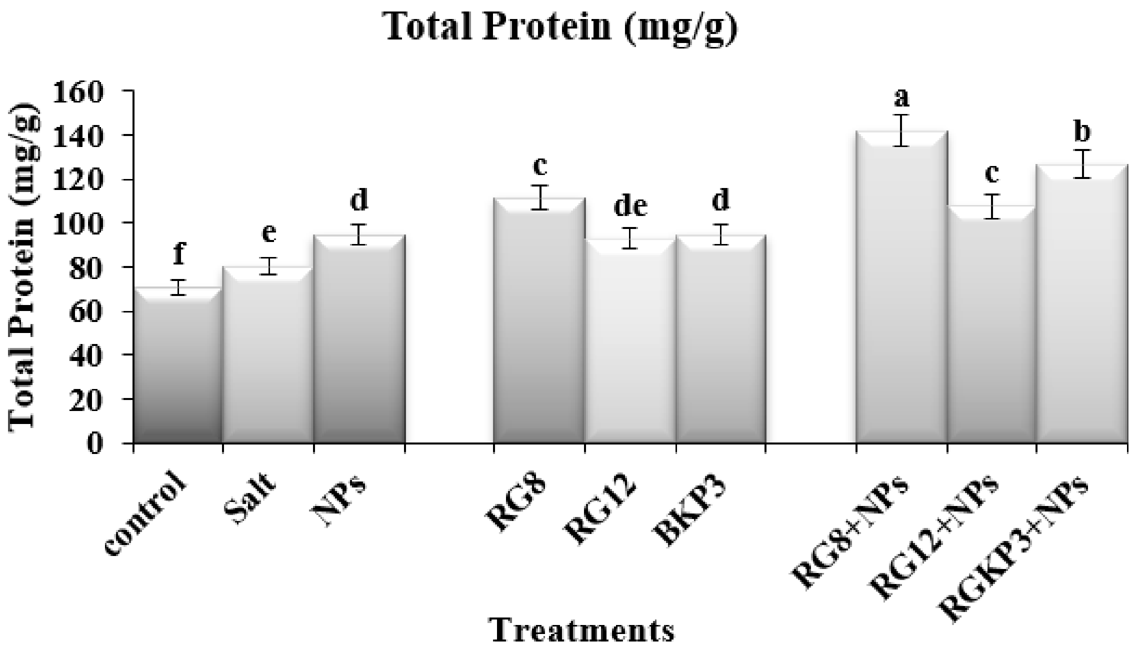


Figure 7.8: Total Protein Content Estimation in Treated Plants from the Pot Experiment focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

7.2 Effect of PGPR (RG8, RG12 and RGKP3) with ZnO NPs (400 ppm) on plant growth and development

Figure 7.9: Groundnut Plants with different treatments in pot experiment after 1 month of in vitro experiment with combined application of three potent PGPR (RG8, RG12 and RGKP3) strains with ZnO NPs

The study evaluated the effects of combining PGPR strains (*Pseudomonas songnenensis*, *Bacillus haynesii*, and *Priestia megaterium*) with chemically synthesized ZnO NPs at an optimized concentration of 400 ppm on the growth and development of groundnut plants. Compared to untreated plants, as well as those treated only with ZnO NPs, only PGPR, or zinc salt, the combination of PGPR (RG8, RG12, and RGKP3) with 400 ppm ZnO NPs significantly enhanced physicochemical properties and biochemical content in groundnut plants. Among the strains, *Priestia megaterium* (RGKP3) and *Pseudomonas songnenensis* (RG8) were more effective in supporting plant growth and development compared to *Bacillus haynesii* (RG12).