CHAPTER:6

Synergistic Effects of PGPRs and Optimized ZnO Nanoparticles in Pot Experiments

6.1 Effect of PGPR and ZnO NPs on plant growth

This experiment investigates the synergistic effects of three potent PGPR strains combined with ZnO NPs at optimized concentrations on groundnut plant growth. The study aims to explore the interactive role of biological and nanotechnological interventions in enhancing agricultural output.

6.1.1 Physical parameters

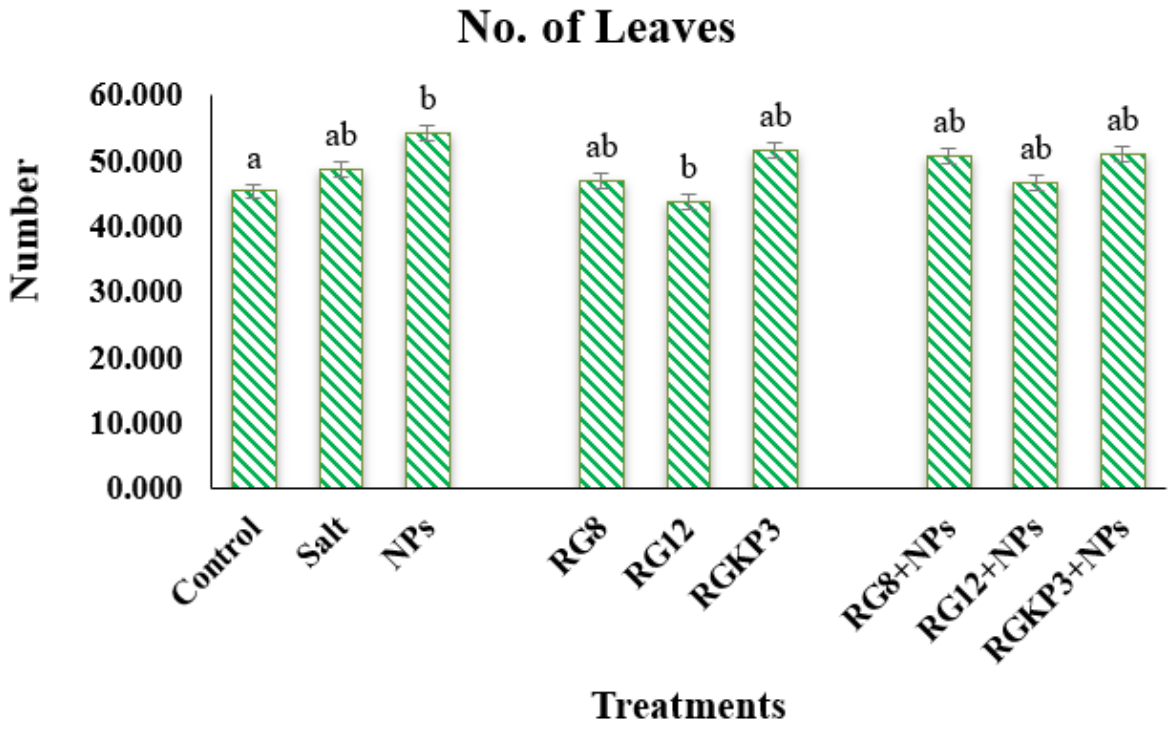

Figure 6.1: Physical parameters number of leaves of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method compared the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figure 6.1 highlights the number of leaves produced under various treatments, demonstrating the impact of different interventions on plant growth. The untreated control plants exhibited the lowest leaf count, with 45 leaves. A slight improvement was observed in the plants treated with RG8, which produced approximately 47 leaves, and those treated with RGKP3, which showed a modest increase to about 51 leaves. The most significant enhancement was seen in the combination treatment of RGKP3 with nanoparticles (NPs), where the leaf count rose to approximately 57. This represents a substantial improvement compared to the control and other individual treatments. These results suggest that the combination of RGKP3 and NPs has a synergistic effect, boosting leaf production more effectively than the treatments applied separately.

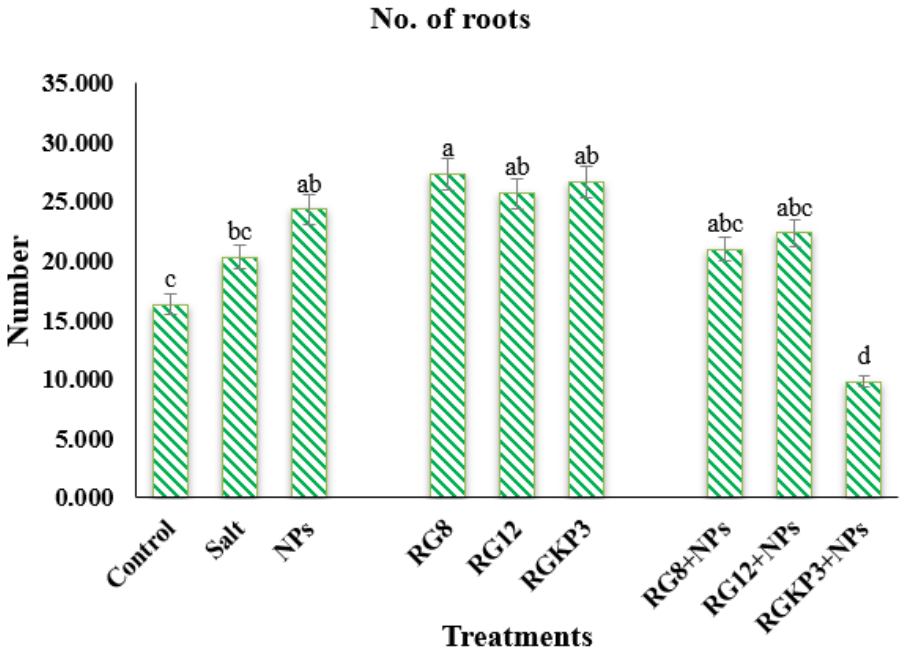

Figure 6.2: Physical parameters number of roots of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method compared the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Isolate RG8 demonstrated the highest root count (~27) among all treatments, highlighting its significant potential for promoting root growth. Only treated with 400 ppm ZnO NPs also resulted in a high number of roots (~24). These combination treatments significantly

enhance nuber of roots parameter compared to the untreated plants (~16).

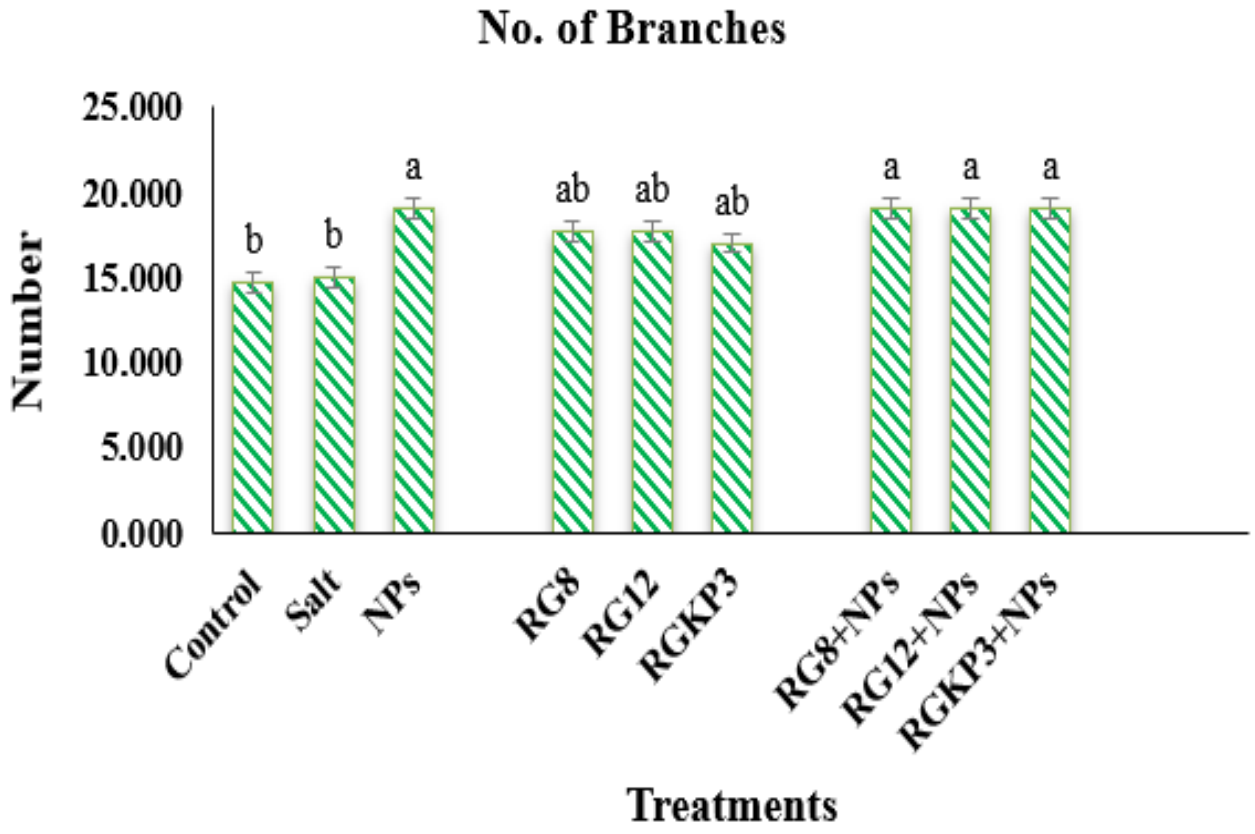

Figure 6.3: Physical parameters number of branches of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method compared the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figure 6.3 shows the effect of various treatments on branch production in plants. Treatments NPs and their combinations PGPR strains such as RG8, RG12, and RGKP3 showed a significant increase in the number of branches. On average, these treatments produced about 19 branches per plant, which is much higher than the untreated control plants that had only 14 branches. The zinc salt treatment also resulted in fewer branches compared to the NPs and PGPR combinations. This clearly shows that using NPs along with PGPR is an effective way to promote branch growth. These results highlight the potential benefits of PGPRs and ZnO NPs compared to other treatments.

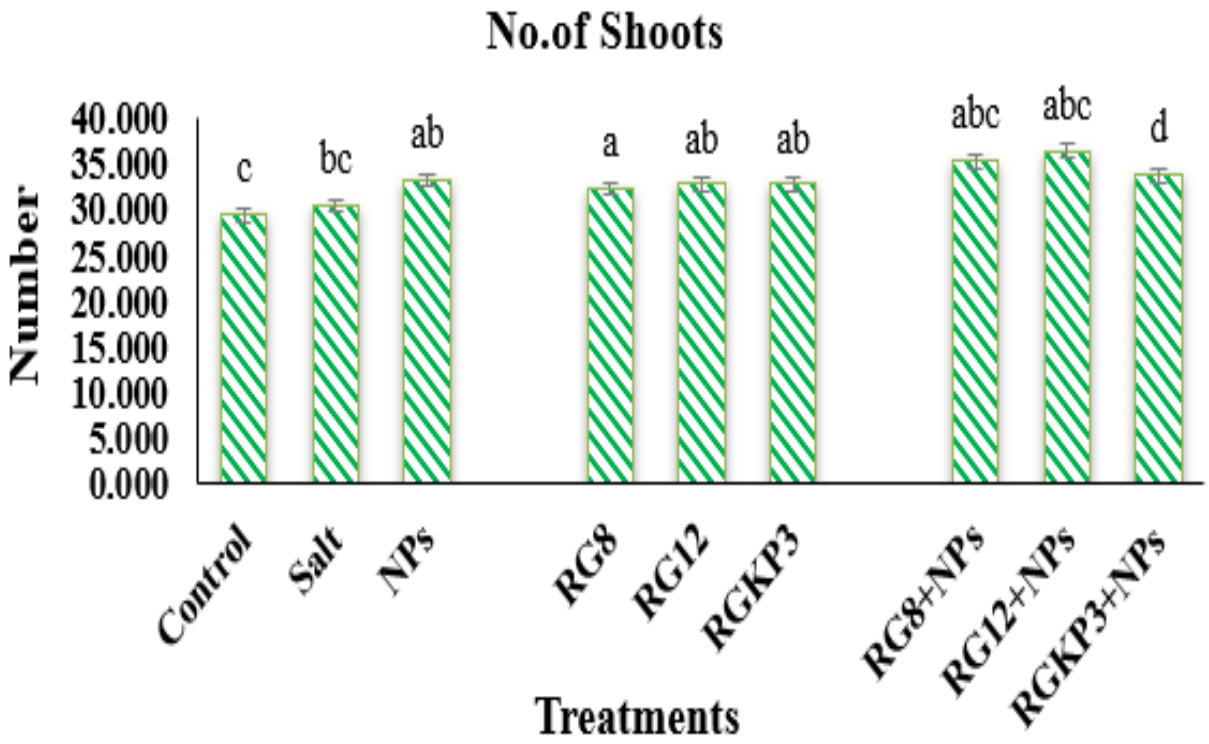

Figure 6.4: Physical parameters number of shoots of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figure 6.4 shows the effects of various treatments on shoot production. Control (untreated) and zinc salt treatments resulted in the lowest shoot counts (~30), while NPs slightly improved shoot numbers (~32). PGPRs, especially RGKP3, showed significant improvements with highest count. Combined treatments, particularly RGKP3+NPs, exhibited the most significant increases, suggesting a synergistic effect.

Figure 6.5 shows the effects of various treatments on root development. The control (untreated) had a root length of 12.6 cm, while zinc salt reduced it to 9.6 cm. NPs slightly increased root length to 12.0 cm. RG8 showed a slight increase (11.8 cm), and RG12 caused a moderate root length (10.5 cm). RGKP3 showed the highest improvement with 15.8 cm root length. Combined treatments did not significantly accomplish individual treatments.

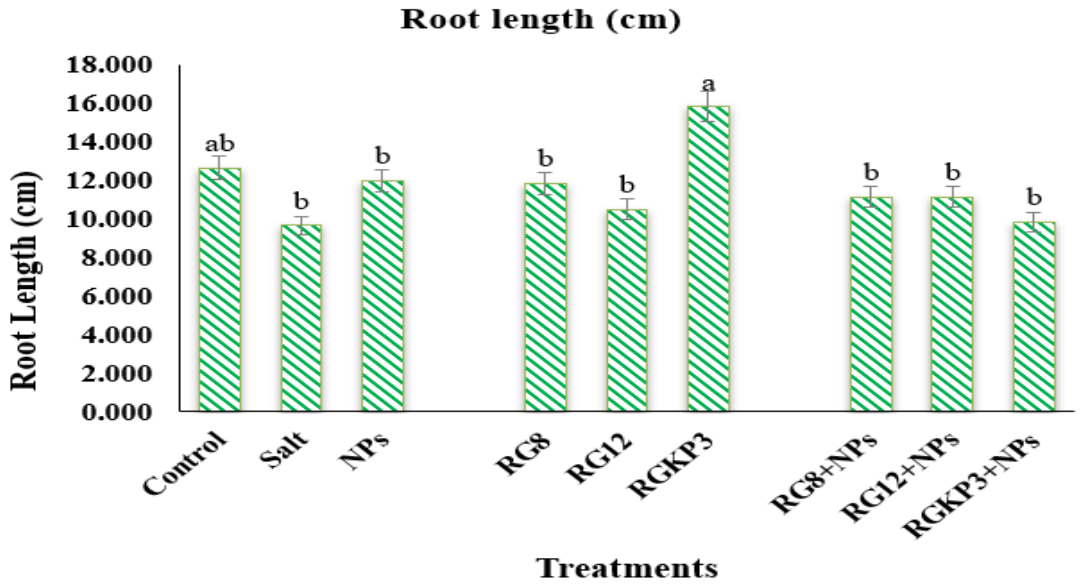

Figure 6.5: Measurement of root length(cm) of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

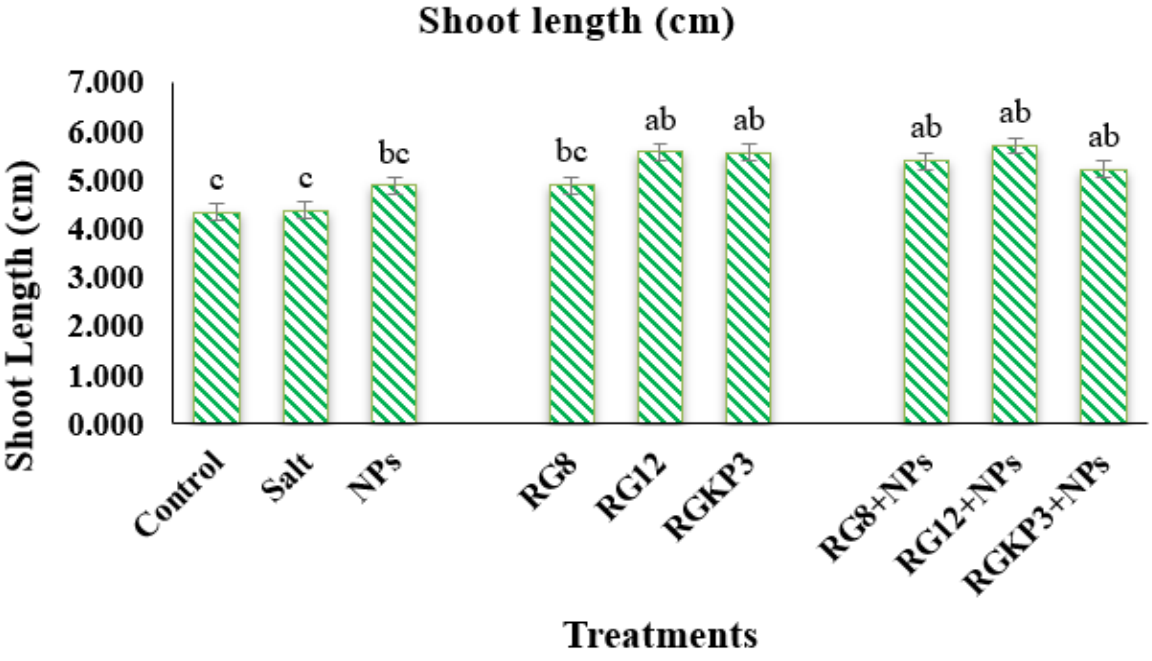

Figure 6.6: Measurement of shoot length(cm) of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt and control (untreated) focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figure 6.6 shows that control and zinc salt treatments had no significant effect on "Shoot Length." NPs slightly increased shoot length, while RGKP3 significantly enhanced shoot growth. Combined treatments showed moderate improvements, but RGKP3 was the most effective, followed by RG12 and RG8.

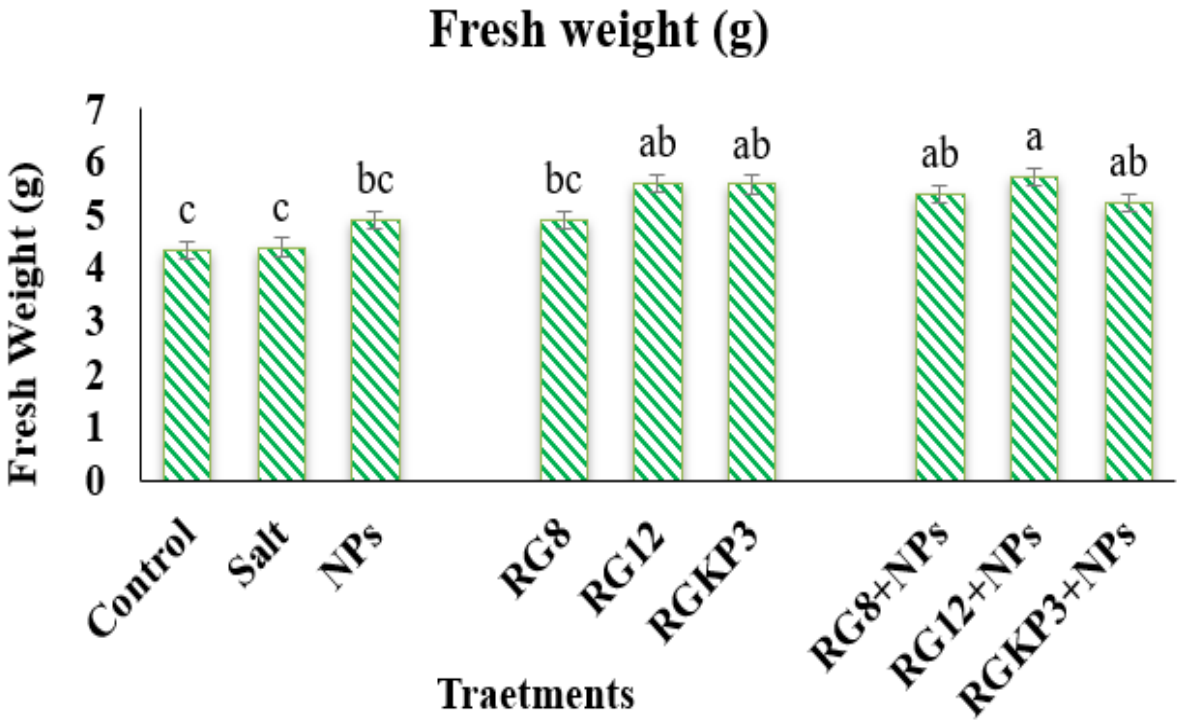

Figure 6.7: Measurement of fresh weight(g) of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

Figures 6.7 and 6.8 demonstrate the effects of various treatments on the fresh and dry weights (g) of plants, revealing that the combination of PGPRs, specifically RG8, RG12, and RGKP3 with NPs significantly enhances plant biomass. This combined treatment proved to be the most effective among those tested, suggesting a synergistic interaction between PGPRs and ZnO NPs. The enhanced biomass observed under this treatment suggests that the integration of these PGPRs with NPs effectively boosts nutrient uptake, stimulates growth-promoting processes, and strengthens the plant's overall development. These findings highlight the potential of such combined treatments as an advanced strategy for maximizing plant biomass and productivity.

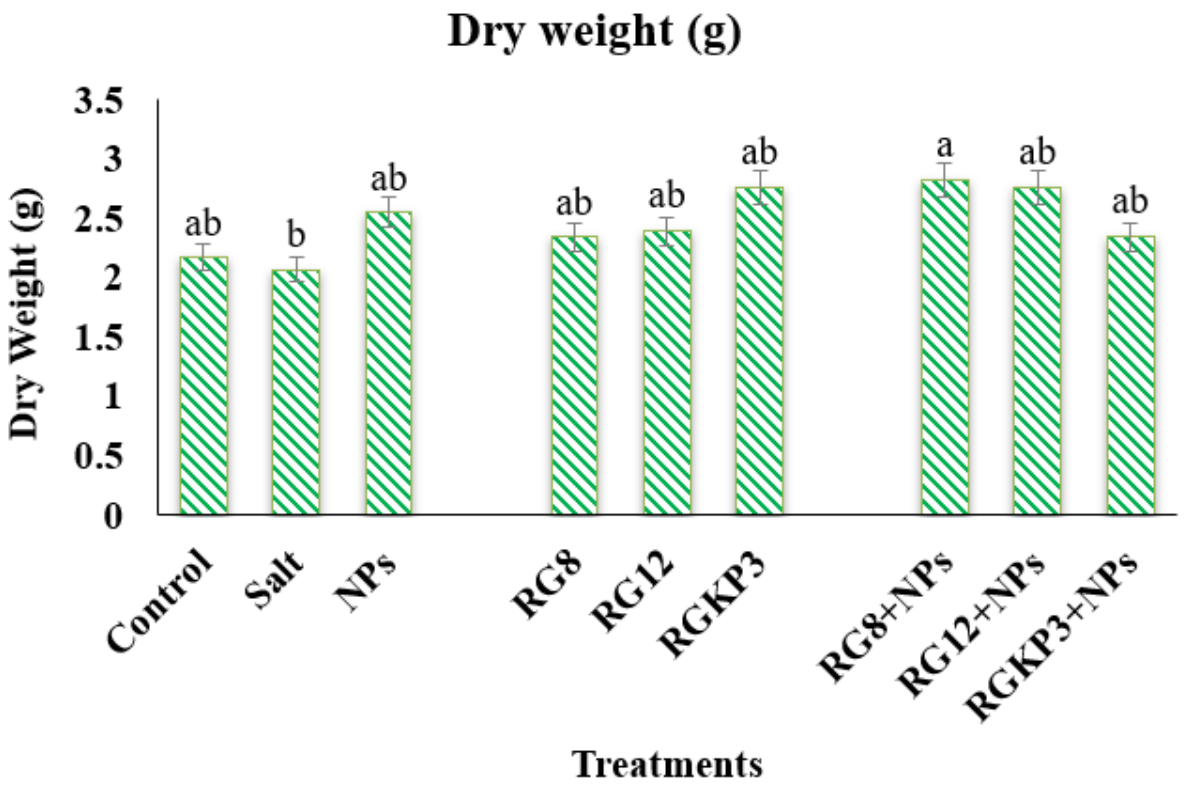

Figure 6.8: Measurement of dry weight(g) of plants with combined PGPR and ZnO NPs, Only PGPRs, Only NPs, zinc salt, and control (untreated) focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

The improvements were particularly pronounced in fresh weight, indicating enhanced growth-promoting effects potentially linked to increased water retention, nutrient uptake, or metabolic activity. The increased dry weight further emphasizes the positive impact of these treatments on overall biomass and plant health.

6.1.2 Biochemical parameters

Biochemical parameters for all treated plants, including those treated with only ZnO NPs, salt of ZnO, only PGPR, and a combination of PGPR with NPs, as well as control (untreated) plants, were assessed in vitro. This approach was based on the methodology used in a previous pot experiment to ensure accuracy and reproducibility. The biochemical analysis aimed to evaluate various key indicators of plant health and growth, providing a comprehensive understanding of the effects of each treatment on the plants physiological responses.

6.1.2.1 Estimation of Total Chlorophyll content for RG8 (*Pseudomonas songnenensis*)

Figure 6.9 shows total chlorophyll levels under different treatments. The control (untreated) group has the lowest content (11.25 mg/g), while zinc salt (14.98 mg/g) and ZnO NPs (18 mg/g) show moderate increases. RG8 (22.06 mg/g) significantly enhances chlorophyll, and RG8+NPs (23.80 mg/g) achieves the highest level with a synergistic effect. RG8 and RG8+NPs are the most effective treatments.

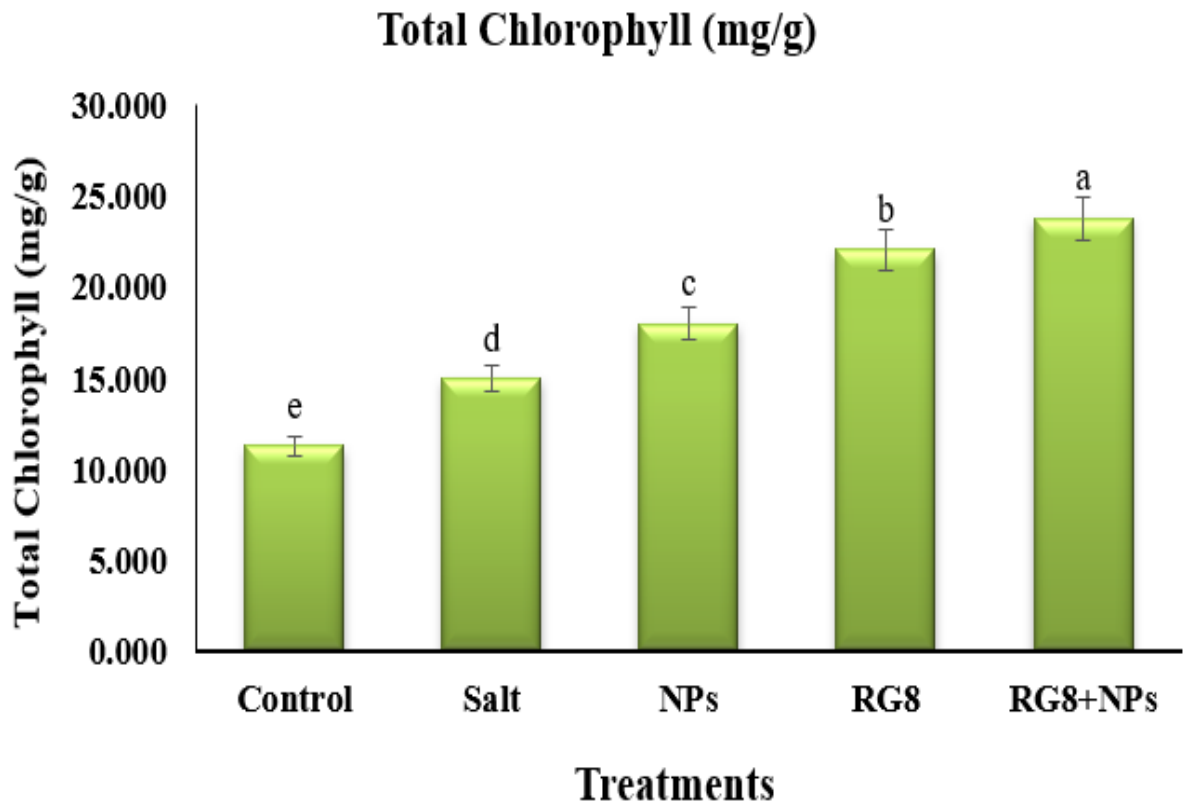

Figure 6.9: Total chlorophyll content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.2 Estimation of Carotenoid content for RG8 (*Pseudomonas songnenensis*)

Figure 6.10 shows carotenoid levels under various treatments. The control (untreated)

group has the lowest concentration (34.45 µg/g), while zinc salt (54.18 µg/g) and ZnO NPs (61.30 µg/g) moderately increase levels. RG8 (68.85 µg/g) significantly enhances carotenoid, and the RG8+NPs combination (72.98 µg/g) achieves the highest levels, showing a synergistic effect. RG8 and RG8+NPs are the most effective treatments compared to other treatments.

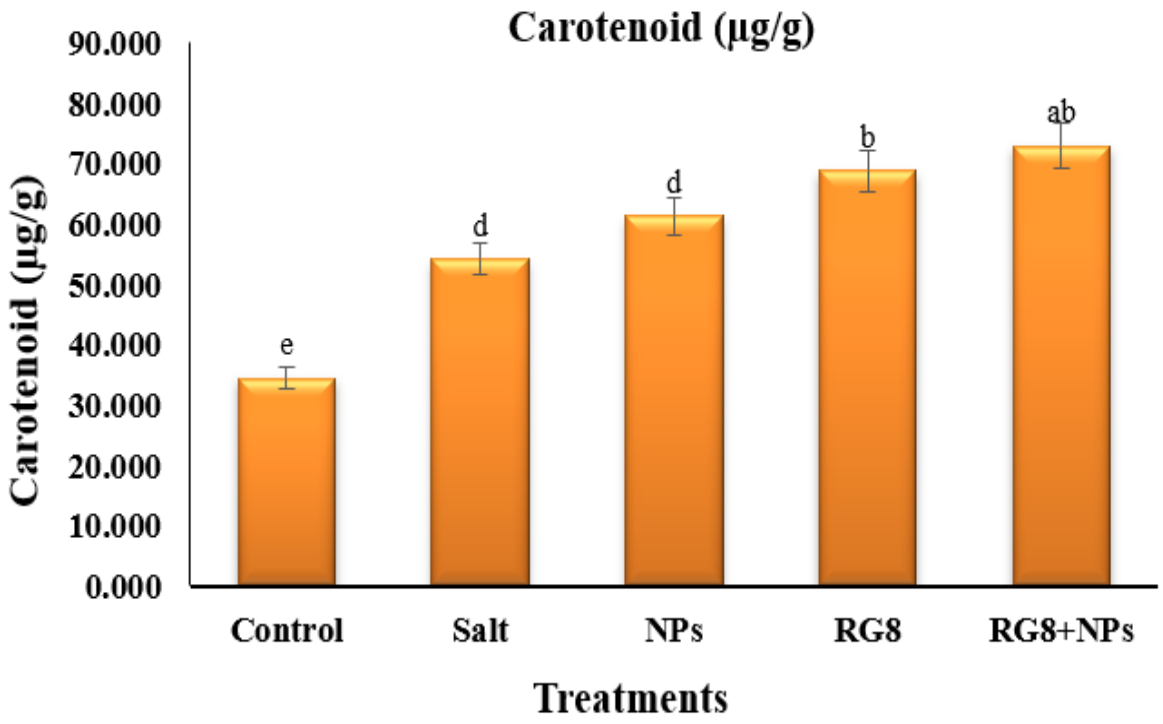

Figure 6.10: Carotenoid content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.3 Estimation of Flavonoid content for RG8 (*Pseudomonas songnenensis*)

Figure 6.11 shows flavonoid content (µg/g) across treatments, highlighting significant increases under specific interventions. The control (untreated) group exhibited the lowest flavonoid level (1.72 µg/g), while zinc salt moderately increased it to 1.96 µg/g, suggesting a defensive response. These results provide a clear contrast between the untreated control and the zinc salt treatment, highlighting the latter's modest yet measurable impact on flavonoid levels.

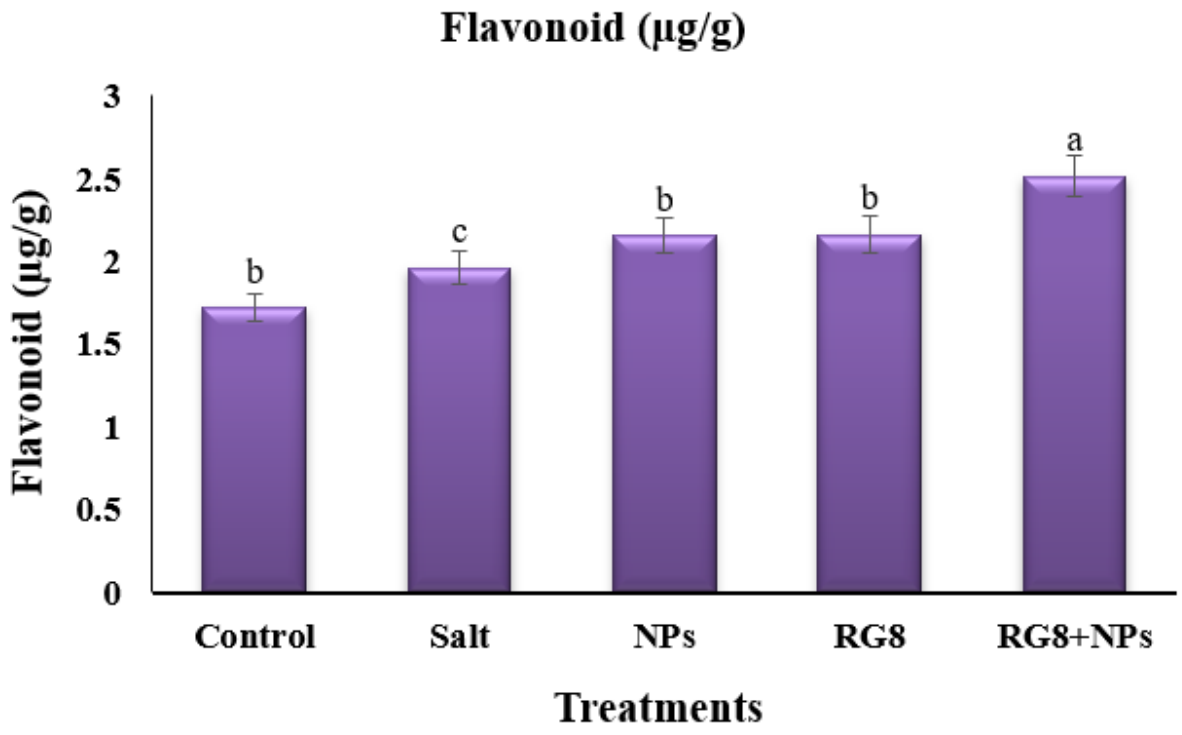

Figure 6.11: Flavonoid content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

NPs and RG8 treatments independently enhanced flavonoid production to comparable levels (2.15 µg/g and 2.16 µg/g, respectively), indicating their positive influence on metabolic activity. The combination of RG8 and NPs resulted in the highest flavonoid content (2.513 µg/g), demonstrating a synergistic effect.

6.1.2.4 Estimation of Proline content for RG8 (*Pseudomonas songnenensis*)

Proline, an amino acid, is essential in plants. It protects plants from various stressors and also aids plant recovery from stress (Ghosh et al., 2022). Figure 6.12 revealed that proline content (mg/g) under different treatments, highlighting its role as a stress marker. The control (untreated) group exhibited the highest proline level (100 mg/g), while salt treatment slightly reduced it to 80 mg/g, indicating an imbalance of proline under stress. NPs and RG8 combined treatments further decreased proline levels to 52 mg/g, suggesting enhanced stress management.

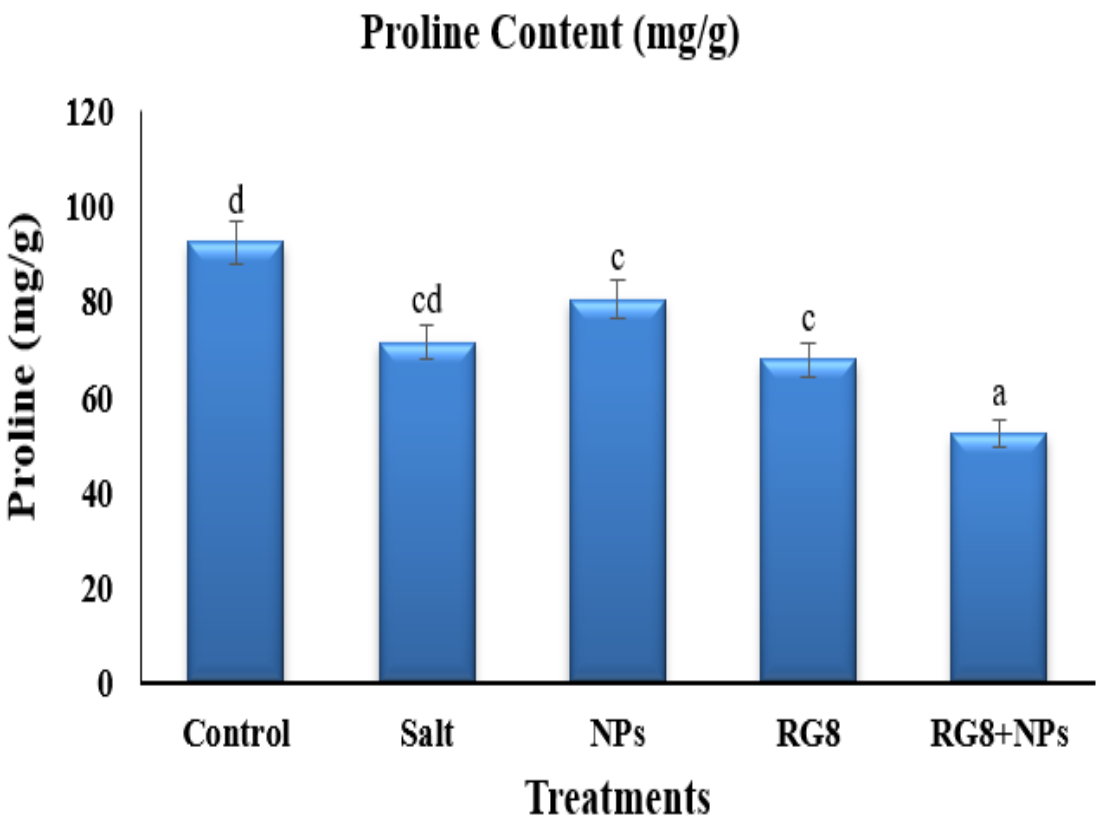

Figure 6.12: Proline content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.5 Estimation of Protein content for RG8 (*Pseudomonas songnenensis*)

Plant proteins play crucial roles in various enzymatic, structural, and functional processes, while also serving as reserves to support the growth and nutritional needs of developing seedlings (Dunwell et al., 2000). Figure 6.13 presents the differences in total protein content (mg/g) across various treatments. The control (untreated) group had the lowest protein level (70.4 mg/g), with zinc salt treatment shows a moderate increase (80.2 mg/g). NPs raised the protein content further to 94.6 mg/g, while RG8 treatment led to a more significant increase (111.4 mg/g). The highest protein content (142 mg/g) was observed with the combination of RG8+NPs, indicating a synergistic effect.

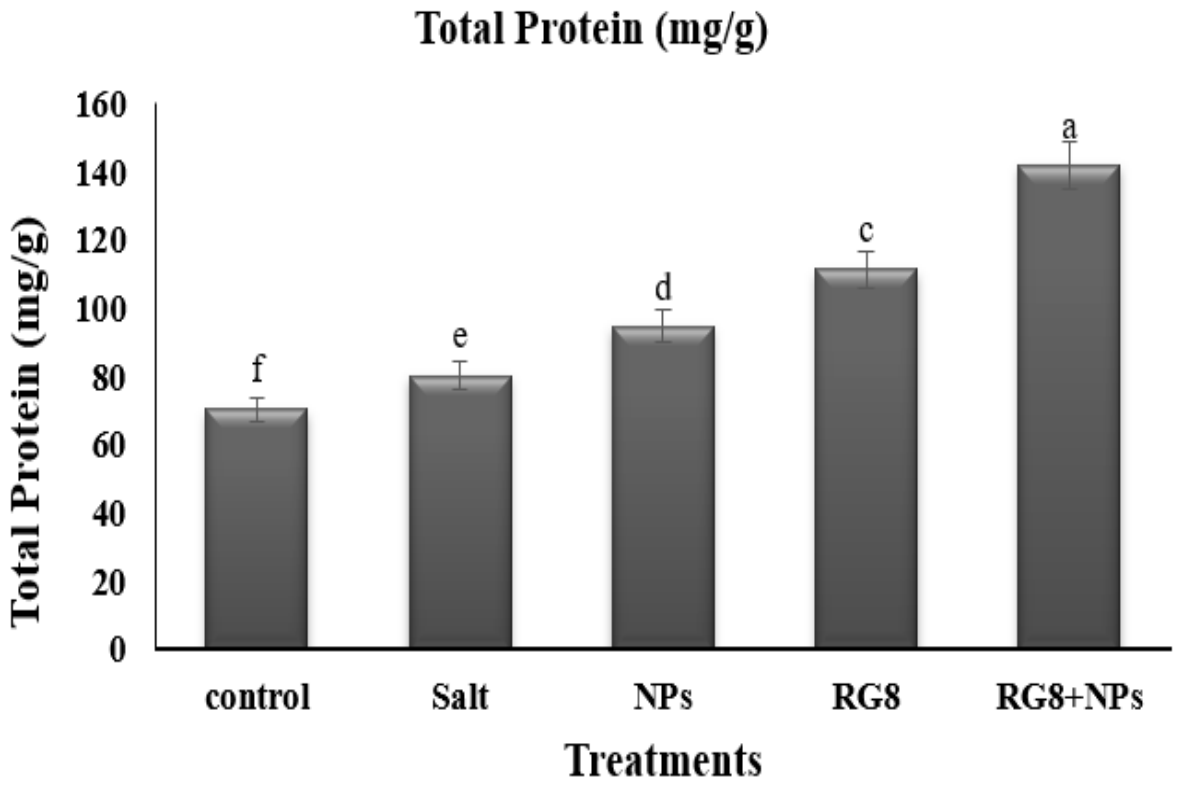

Figure 6.13: Total protein content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.6 Estimation of Total Sugars content for RG8 (*Pseudomonas songnenensis*)

Sugars play a crucial role in various stages of the plant life cycle, interacting with signaling molecules such as phytohormones to regulate growth and development (Mishra et al., 2022). Figure 6.14 shows the total sugar content (mg/g) under different treatments. The control (untreated) group had the lowest sugar level (109.19 mg/g), while zinc salt treatment showed a modest increase (122.90 mg/g). NPs raised the sugar content to 132.09 mg/g, and RG8 further increased it to 140.65 mg/g. The combination of RG8+NPs resulted in the highest sugar content (144.19 mg/g). These results highlight that NPs and RG8+NPs are the most effective treatments for enhancing sugar content in plants.

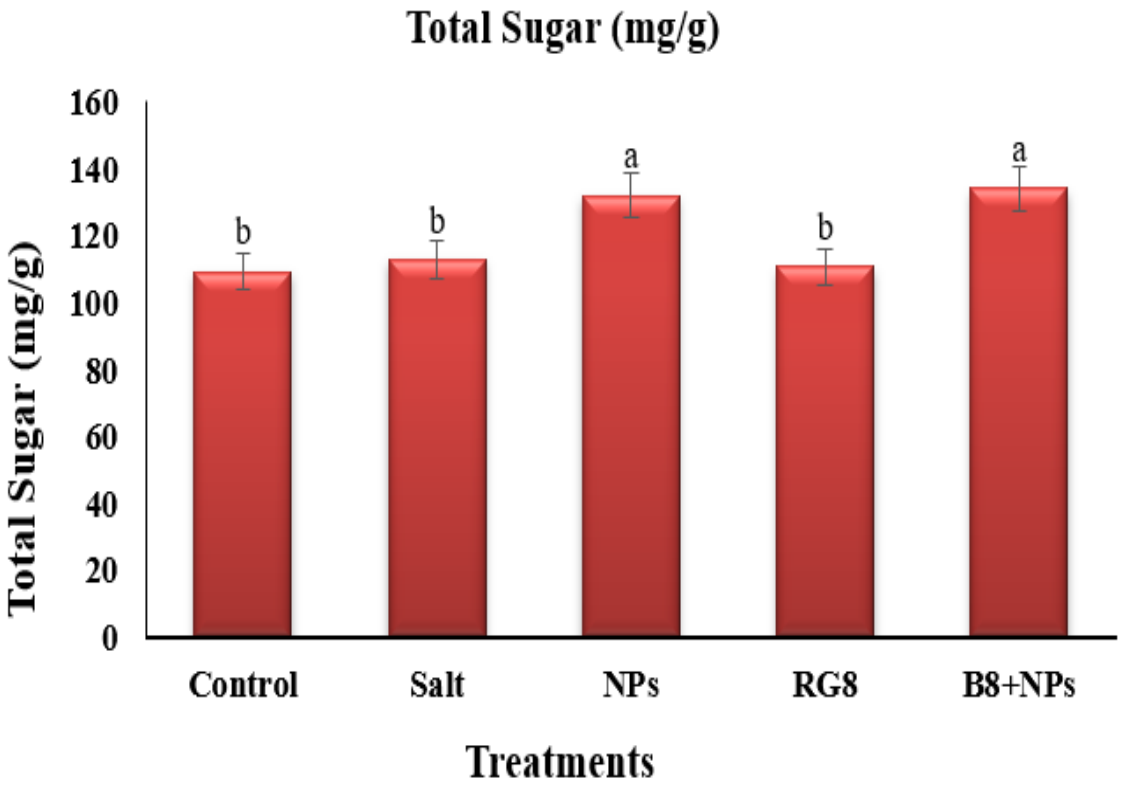

Figure 6.14: Total sugar content estimation for various treatments, including RG8 (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.7 Estimation of Total Chlorophyll content for RG12 (*Bacillus haynesii*)

Treatment with RG12 and NPs resulted in the highest chlorophyll content (24.12 mg/g), significantly higher than all other treatments, including control (untreated) (11.25 mg/g), zinc salt (14.98 mg/g), and NPs or RG12 alone. The combined treatment of RG12 and NPs demonstrated a synergistic effect, significantly enhancing chlorophyll production compared to the other groups (fig 6.15). This suggests that integrating NPs with PGPR like RG12 can effectively improve photosynthetic efficiency, as seen in the enhanced chlorophyll content. Additionally, *Pseudomonas fluorescens* strains elevated chlorophyll fluorescence and chlorophyll pigments in sweet maize under water-deficit stress (Zarei et al. 2020).

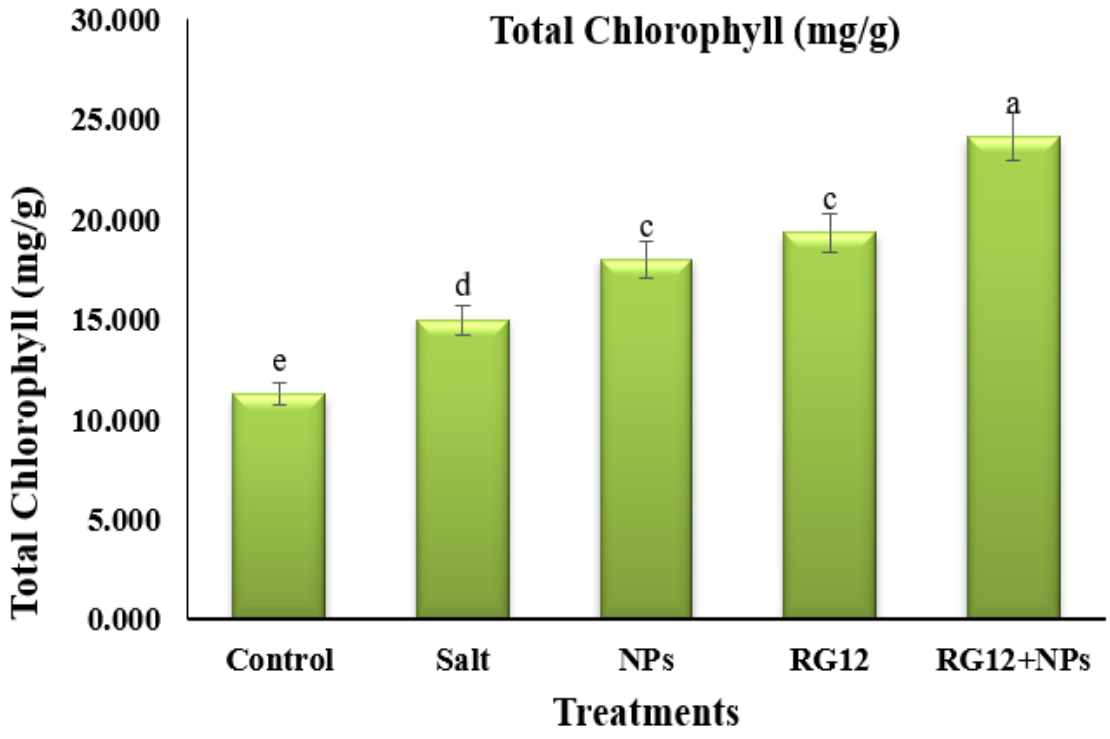

Figure 6.15: Total chlorophyll content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.8 Estimation of Carotenoid content for RG12 (*Bacillus haynesii*)

The control (untreated) group has the lowest carotenoid content (34.453 µg/g), significantly lower than all other treatments. Zinc salt-treated plants show a moderate increase in carotenoid levels, while NPs further improve content. Figure 6.16 displays the production of carotenoid content across various treatments, including control (untreated), salt, NPs, RG12, and a combination of RG12 and NPs. RG12 treatment enhances carotenoid production to 67.893 µg/g, a significant increase compared to salt and NPs alone. The combination of RG12 and NPs produces the highest carotenoid levels at 75.627 µg/g, demonstrating a synergistic effect. These findings indicate that NPs and RG12, particularly in combination, are the most effective treatments for boosting carotenoid production.

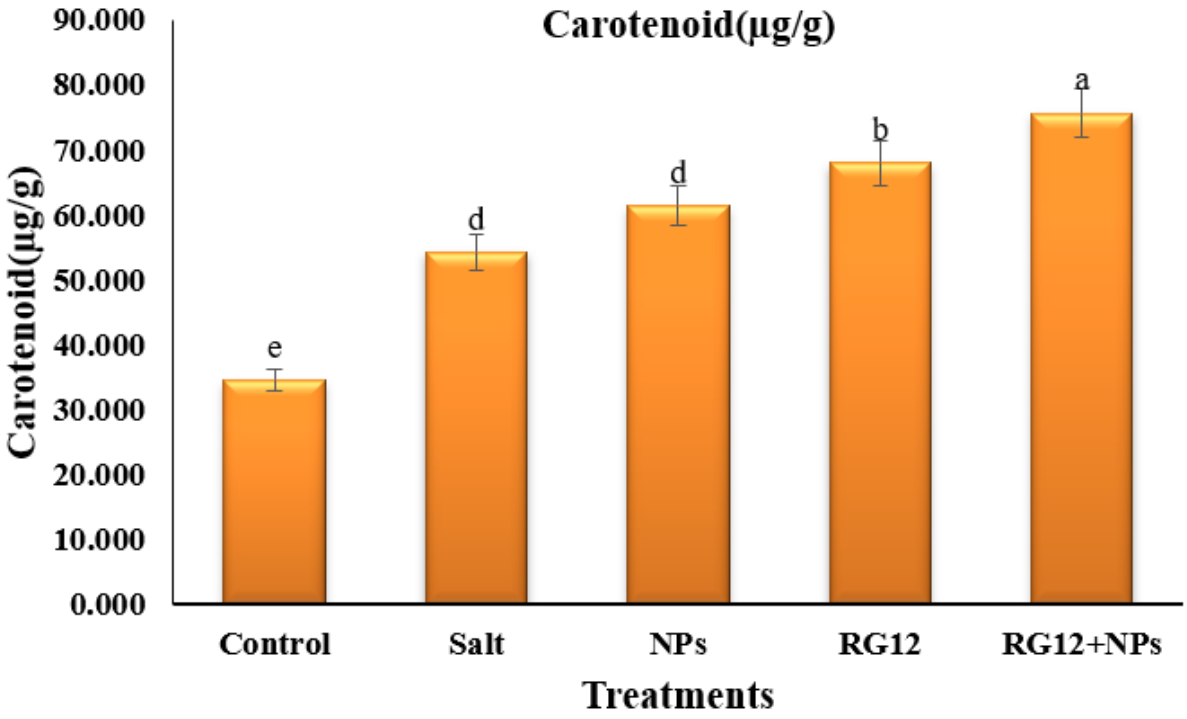

Figure 6.16: Carotenoid content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.9 Estimation of Flavonoids content for RG12 (*Bacillus haynesii*)

Flavonoids have several roles in plants, including controlling cell development, attracting pollinators and insects, and defending against biotic and abiotic stressors (Shah and Smith, 2020).

The flavonoid content varied across treatments, with the control (untreated) group showing the lowest levels (1.72 µg/g), slightly increased by zinc salt (fig 6.17). Both NPs and RG12 treatments enhanced flavonoid production compared to the control (untreated) and zinc salt, while the combination of RG12 and NPs resulted in the highest flavonoid levels (2.44 µg/g), indicating a synergistic effect. The functional roles of flavonoids in mitigating abiotic stress, regulating of antioxidant systems, participating in signaling networks, and influencing on various physiological processes within plants (Shomali et al., 2022).

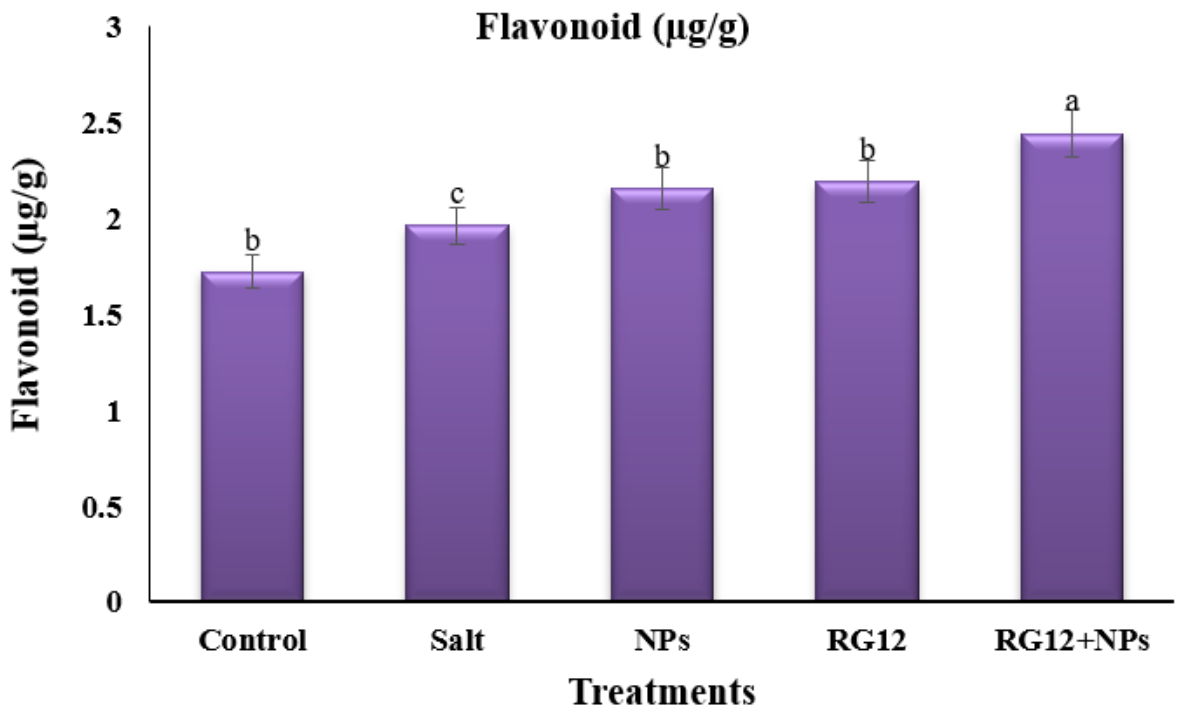

Figure 6.17: Flavonoid content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.10 Estimation proline content for RG12 (*Bacillus haynesii*)

Proline also serves as an osmolyte, dropping reactive oxygen species injuries and functioning as a hydroxyl radical scavenger, protecting cells from stress-induced damage (Hosseinifard et al., 2022). Figure 6.18 shows notable variations in proline content across treatments. Control (untreated) plants had the highest proline levels (96 mg/g), indicating stress. Salt stress slightly reduced proline, while NPs further decreased it to 80 mg/g, suggesting improved plant health. The lowest proline levels were observed in RG12 (55 mg/g) and RG12+NPs treatments (26 mg/g), highlighting their role in stress relief. These results indicate that RG12 and NPs, especially when combined, help reduce the reliance on proline as an osmoprotectant, thereby supporting overall plant health.

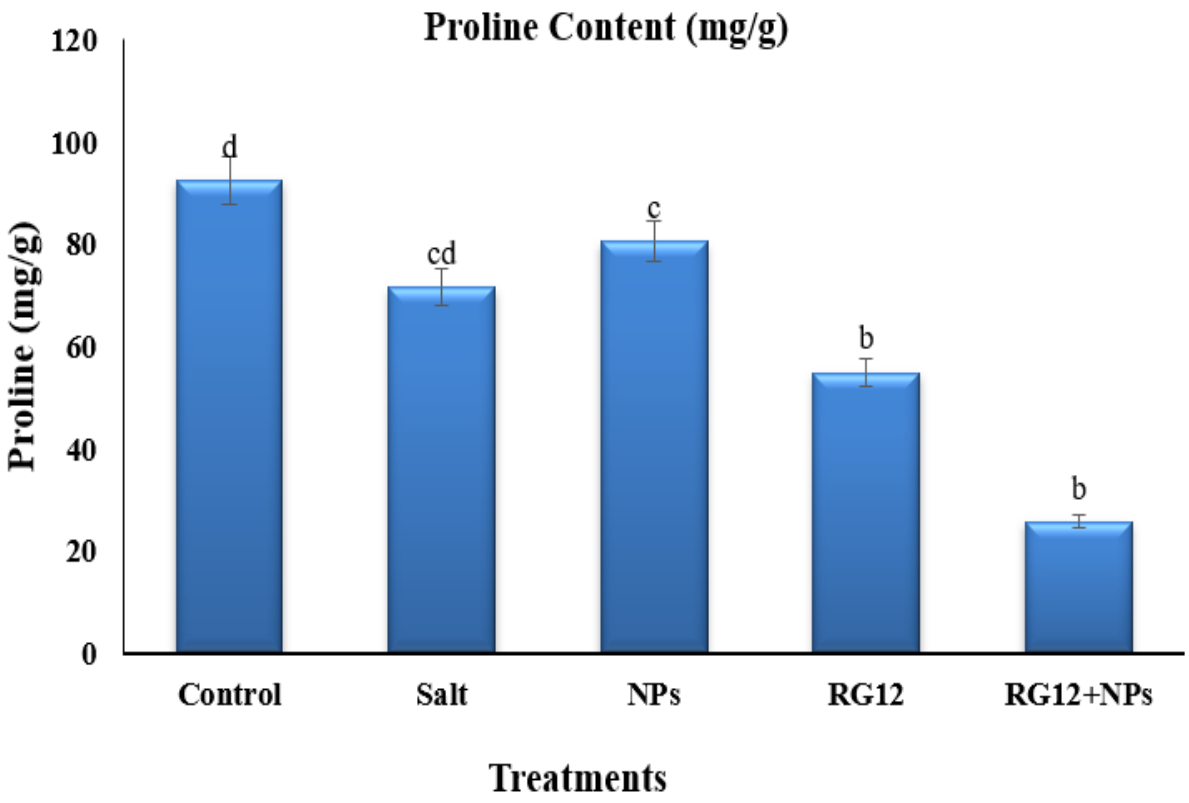

Figure 6.18: Proline content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.11 Estimation of Protein content for RG12 (*Bacillus haynesii*)

Plant proteins play essential roles in enzymatic, structural, and functional processes and also serve as storage for the nutritional needs of developing seedlings (Broadley et al., 2012). Figure 6.19 illustrates total protein content (mg/g) across various treatments: control (untreated), zinc salt, NPs, RG12, and a combination of RG12 and NPs. The control (untreated) group has the lowest protein content (70.4 mg/g), while zinc salt treatment shows a slight increase to 80.2 mg/g. Only NPs and only RG12 treatments significantly improve protein content, with similar enhancements. The combination of RG12 with nanoparticles (NPs) yielded the highest protein content among all treatments, with a recorded level of 107.6 mg/g. The observed boost in protein content under the RG12+NPs

treatment indicates an improved capacity for stress adaptation and enhanced overall metabolic efficiency.

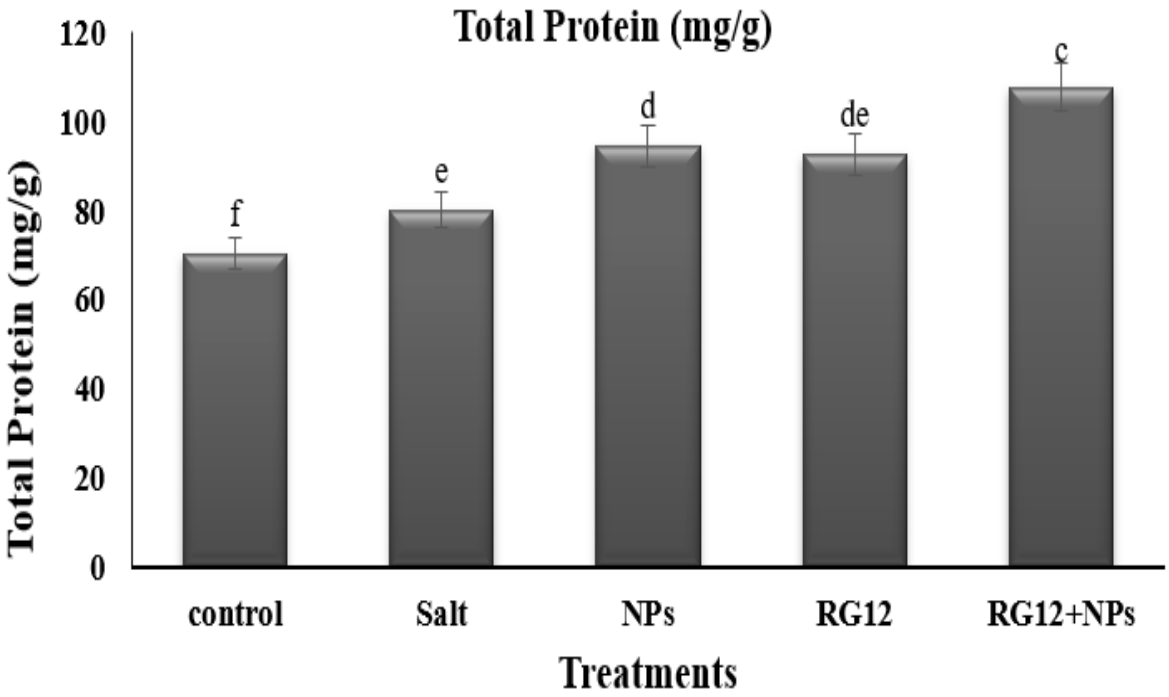

Figure 6.19: Total protein content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.12 Estimation of Total Sugar content for RG12 (*Bacillus haynesii*)

Sugars play a key role in regulating plant growth and development, interacting with phytohormones throughout the plant life cycle (Mishra et al., 2022). The highest total sugar content was observed in treatments with NPs and the combination of RG12+NPs (134 mg/g), highlighting their influence on enhancing sugar metabolism. While RG12 alone had a moderate value (112.41 mg/g), its effect was strengthened when combined with NPs, likely due to improved nutrient uptake and metabolic processes. These findings suggest that NPs and RG12 work together to optimize carbohydrate metabolism, boost energy production, and enhance stress resilience, ultimately improving plant performance under unfavorable conditions.

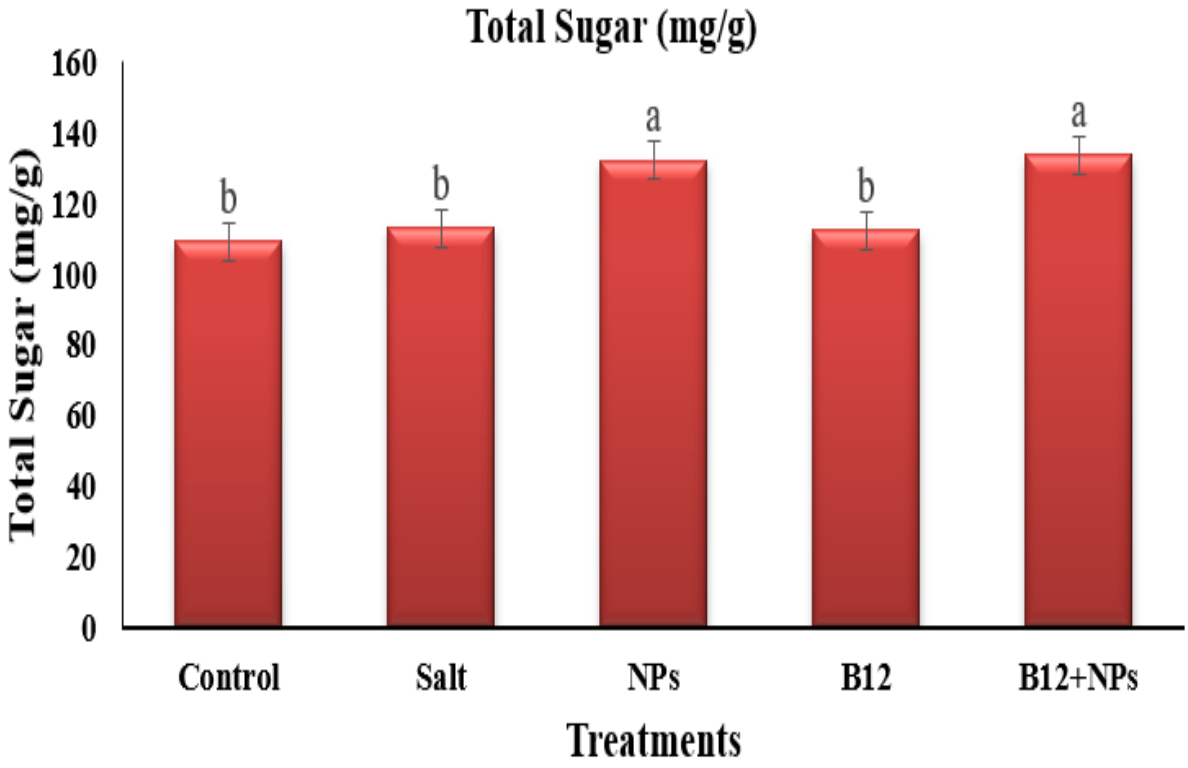

Figure 6.20: Total sugar content estimation for various treatments, including RG12 (*Bacillus haynesii* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.13 Estimation of Total Chlorophyll content for RGKP3 (*Priestia megaterium*)

The control (untreated) group had the lowest chlorophyll level (11.25 mg/g), while the zinc salt treatment showed a slight increase (14.98 mg/g). Both NPs (17.99 mg/g) and RGKP3 (16.28 mg/g) further enhanced chlorophyll content, suggesting better nutrient uptake. The highest chlorophyll content (25.00 mg/g) was observed in the RGKP3+NPs combination, indicating a synergistic effect that improves photosynthesis and overall plant stability. These findings underscore the synergistic advantages of combining RGKP3 with nanoparticles (NPs) in significantly boosting chlorophyll content and improving overall plant productivity. Figure 6.21 provides a detailed comparison of the total chlorophyll content observed across various treatments, highlighting the notable enhancement achieved through the RGKP3 and NP combination.

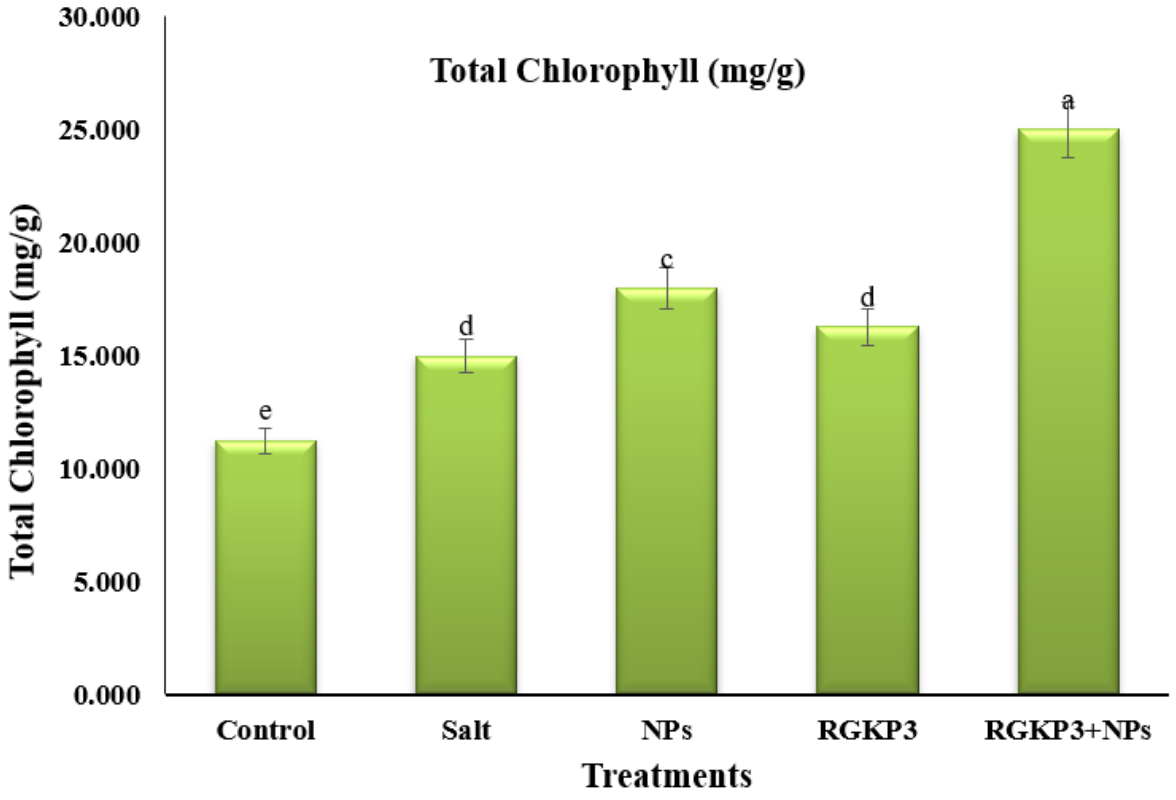

Figure 6.21: Total chlorophyll content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.14 Estimation of Carotenoid content for RGKP3 (*Priestia megaterium*)

The control (untreated) group had the lowest levels (~40 µg/g), while zinc salt treatment increased carotenoid (~55 µg/g). Treatments with NPs (~60 µg/g) and RGKP3 (~65 µg/g) further boosted carotenoid levels, reflecting their roles in enhancing antioxidant activity. The combination of RGKP3 with nanoparticles (NPs) exhibited the highest carotenoid content, reaching approximately 75 µg/g, as demonstrated in Figure 6.22. This significant increase highlights a synergistic effect between RGKP3 and NPs in enhancing carotenoid accumulation. The elevated levels observed in the RGKP3+NPs treatment suggest that this combination effectively strengthens the overall metabolic health. Compared to other treatments, the RGKP3+NPs shows the most effective strategy for boosting carotenoid content, underlining its potential for improving plant resilience and productivity.

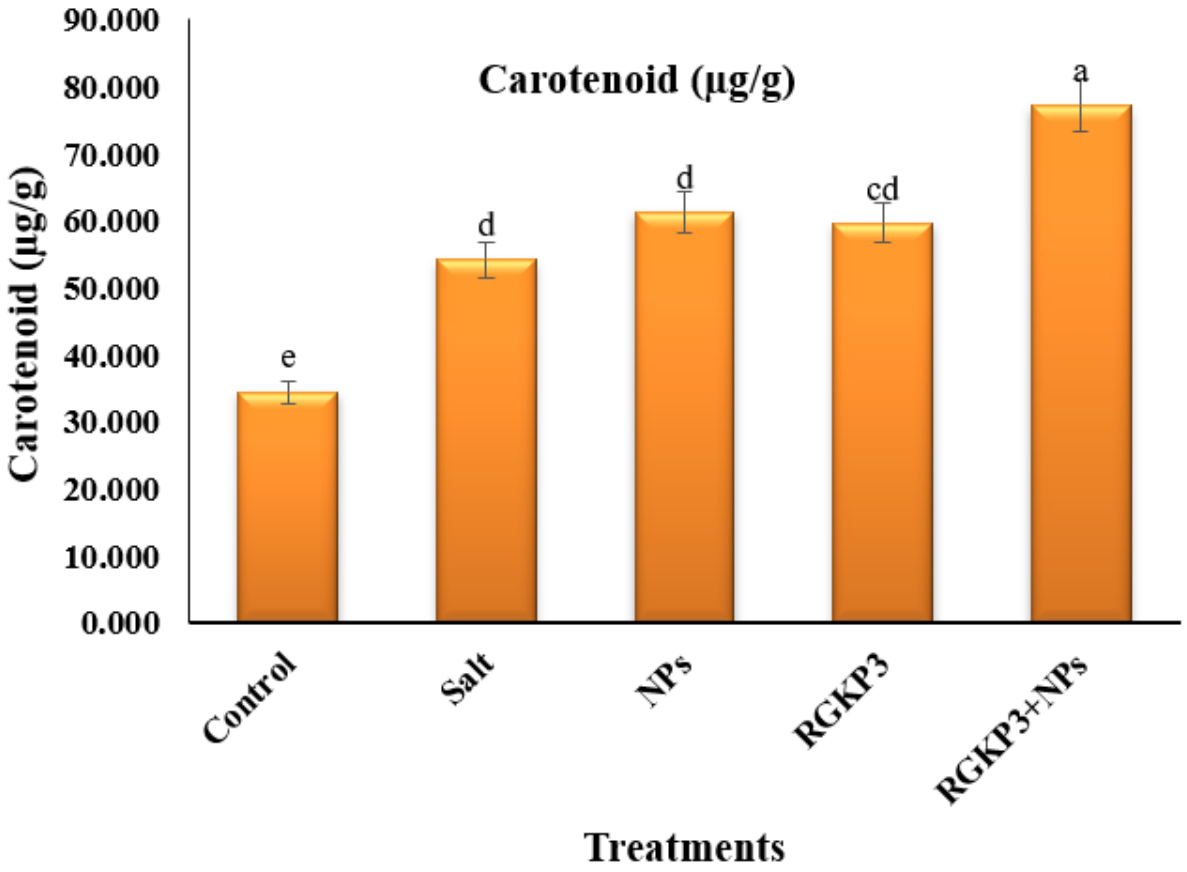

Figure 6.22: Carotenoid content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.15 Estimation of Flavonoid content for RGKP3 (*Priestia megaterium*)

Figure 6.23 shows that the control (untreated) treatment has the lowest flavonoid content (1.72 µg/g), while only 400 ppm NP treatment slightly increases flavonoid levels (2.15 µg/g). Treatments with NPs and RGKP3 individually result in similar increases (2.15 and 2.15 µg/g, respectively), while the RGKP3+NPs combination leads to the highest flavonoid content (2.52 µg/g), suggesting a synergistic effect. This observation indicates that the combination of RGKP3 and zinc oxide nanoparticles (ZnO NPs) significantly enhances flavonoid production in plants. Flavonoids are critical secondary metabolites known for their role in plant defense, acting as antioxidants to mitigate oxidative stress and as protective agents against environmental challenges.

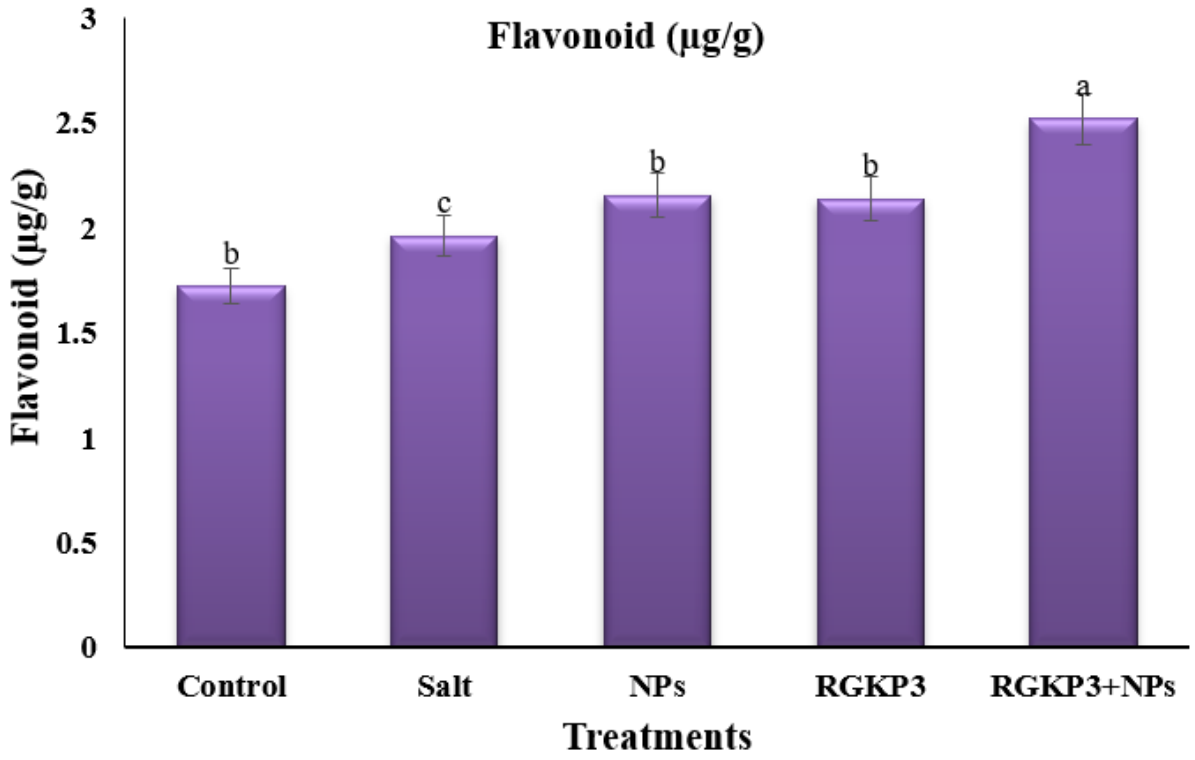

Figure 6.23: Flavonoid content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.16 Estimation of Proline content for RGKP3 (*Priestia megaterium*)

Figure 6.24 presents the proline content across various treatments, illustrating the impact of different treatments on plant stress responses. The control (untreated) group exhibited the highest proline levels (100 mg/g), which can be attributed to the plants natural stress response mechanisms. In contrast, the salt treatment significantly reduced proline content to around 70 mg/g, reflecting the osmotic stress imposed by the salt. However, the combination of RGKP3 and NPs led to the most significant reduction in proline content, lowering the levels to 45 mg/g. This outcome highlights a synergistic interaction between RGKP3 and nanoparticles (NPs), resulting in a significant enhancement of the plant's ability to tolerate stress. The combined treatment demonstrates a more pronounced effect on stress tolerance compared to the application of either RGKP3 or NPs alone.

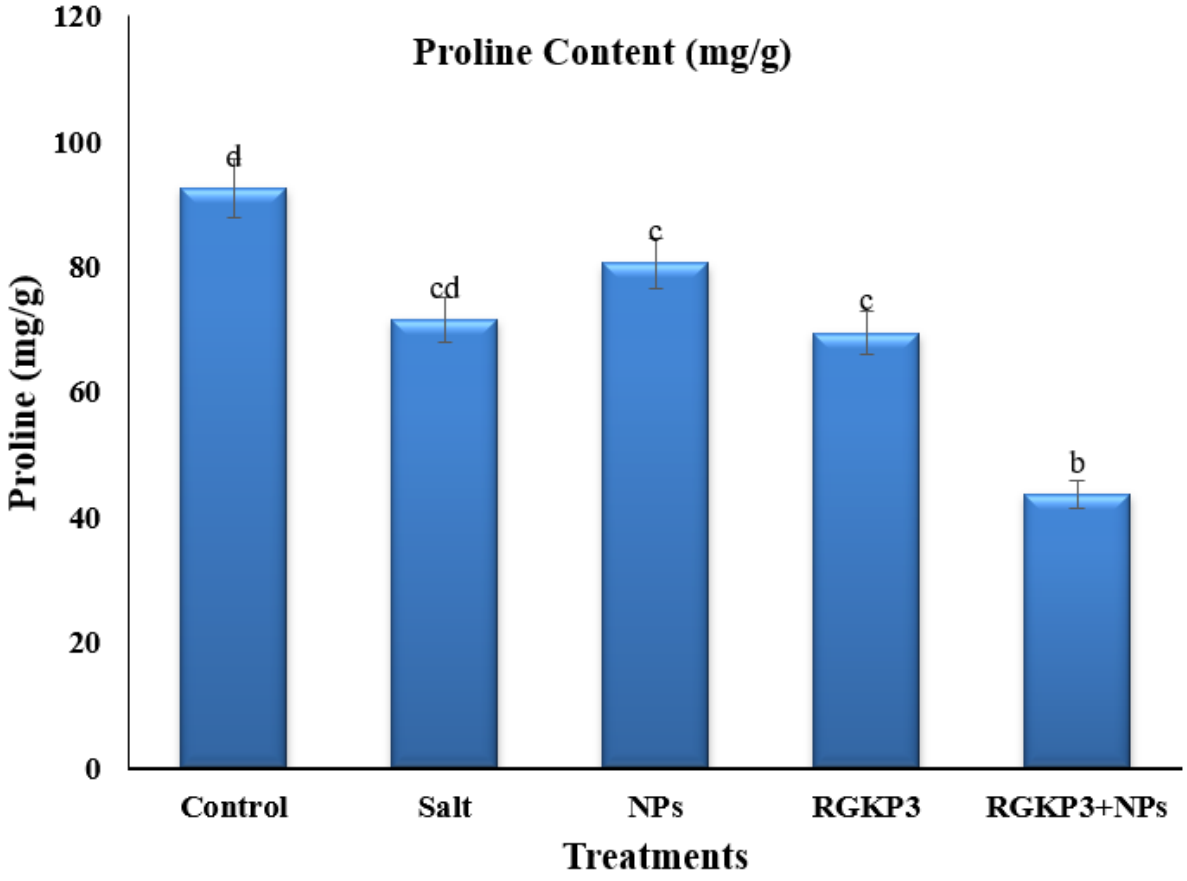

Figure 6.24: Proline content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.17 Estimation of Protein content for RGKP3 (*Priestia megaterium*)

Figure 6.25 illustrates the protein content across different treatments, with the control (untreated) plants showing the lowest protein content at 70.4 mg/g. Treatments with NPs alone (94.6 mg/g) or with the RGKP3 (94.6 mg/g), both resulted in an increase in protein content, While these individual treatments showed improvements, the significant enhancement was observed when RGKP3 was combined with NPs, resulting in a marked increase in protein content to 126.6 mg/g. The combination of NPs and RGKP3 appears to optimize the plants metabolic processes, leading to improved growth and productivity, particularly under unfavorable conditions.

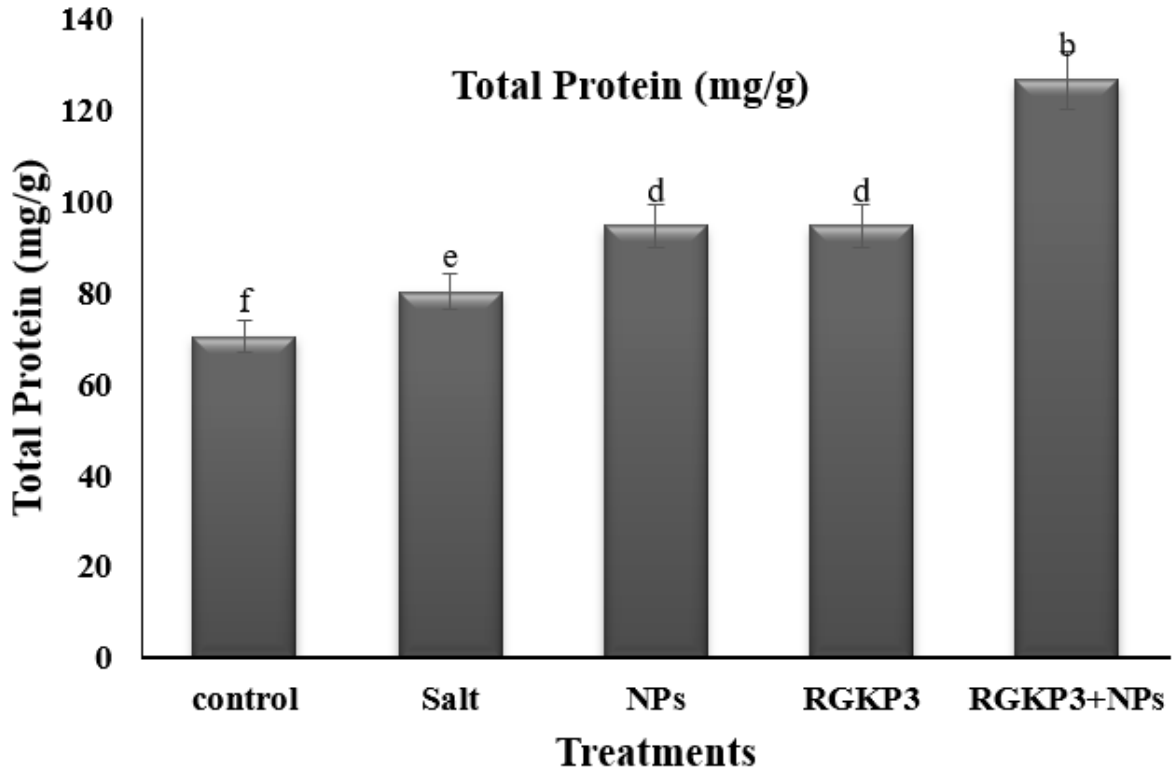

Figure 6.25: Total protein content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

6.1.2.18 Estimation of Sugar content for RGKP3 (*Priestia megaterium*)

Figure 6.26 provides a comprehensive comparison of sugar content across various treatments, including the untreated control, zinc salt, NP, RGKP3, and the combined RGKP3+NPs treatment. The untreated control group exhibits a baseline sugar content of 109 mg/g, while the zinc salt treatment slightly raises it to 112 mg/g, indicating minimal impact. In contrast, NP treatment significantly increases sugar content to 132 mg/g, highlighting its pronounced effect on sugar accumulation. The RGKP3 treatment alone does not yield a notable enhancement, reflecting its limited individual impact. However, the RGKP3+NPs combination boosts sugar content to 126 mg/g, demonstrating a synergistic effect that surpasses the control and zinc salt treatments, though it does not exceed the levels achieved by NPs alone. These results emphasize the differential

influences of the treatments, with NPs and their combination with RGKP3 showing the most substantial potential for enhancing sugar content.

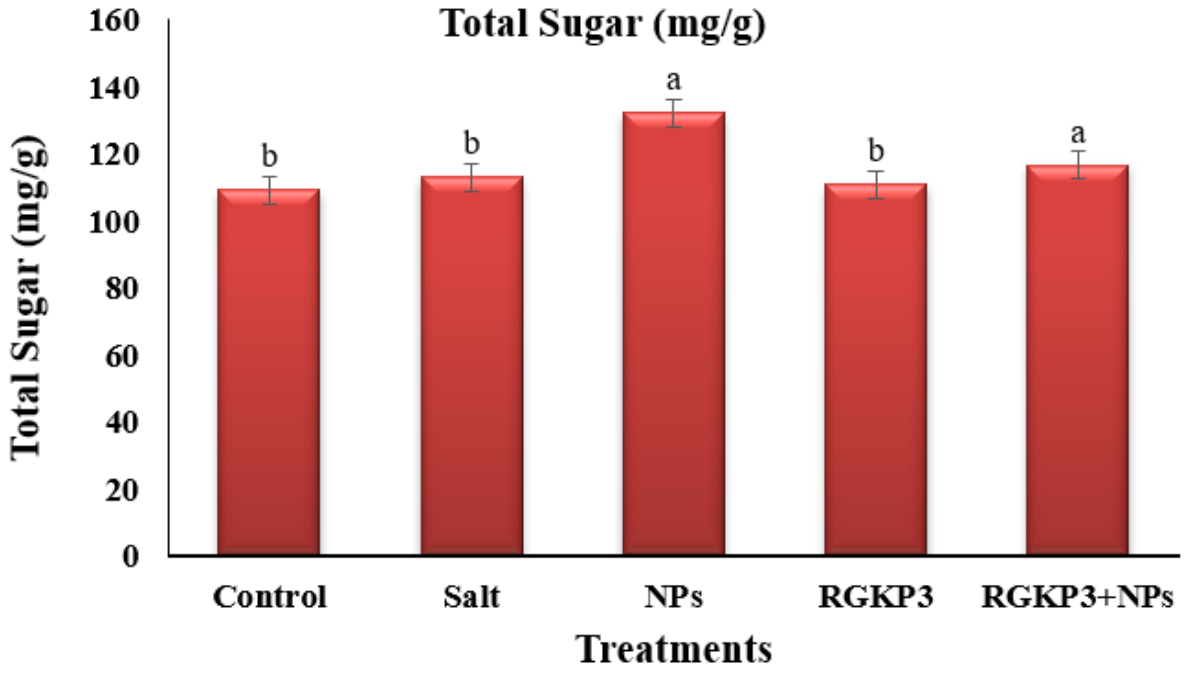

Figure 6.26: Total sugar content estimation for various treatments, including RGKP3 (*Priestia megaterium* with 400 ppm ZnO NPs), zinc salt alone, 400 ppm ZnO NPs alone, and untreated plants, focusing on SSR expression. Duncan's method was used to compare the means at a 1% probability level, with columns sharing the same letters indicating no significant differences

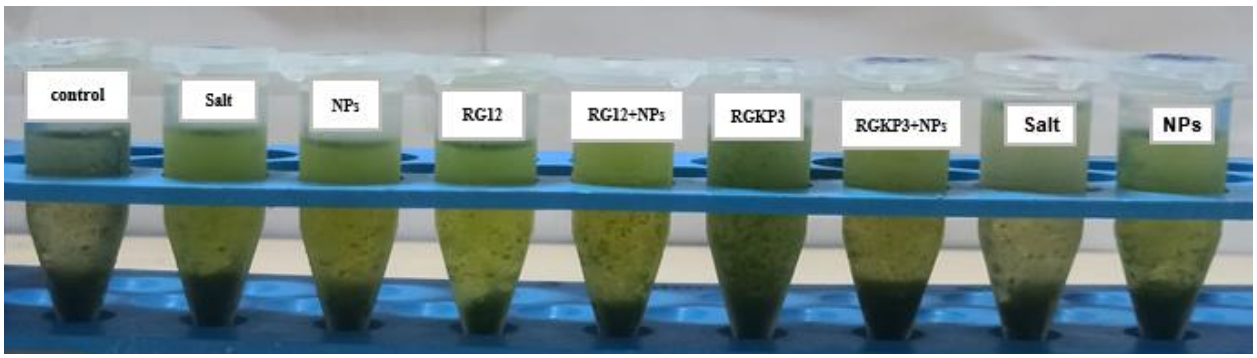

Figure 6.27: Total Chlorophyll and Carotenoid content of all treated plants (Salt=Zinc acetate-400 ppm RG8=*Pseudomonas songnenensis*, RG8+NPs=*Pseudomonas songnenensis* with 400 ppm ZnO NPs, RG12=*Bacillus haynesii*, RG12+NPs=*Bacillus haynesii* with 400 ppm ZnO NPs, RGKP3=*Priestia megaterium*, RGKP3+NPs= *Priestia megaterium with 400 ppm* ZnO NPs

The total chlorophyll and carotenoid content of plants treated with different treatments were evaluated, and their combinations with 400 ppm ZnO NPs (RG8+NPs, RG12+NPs, RGKP3+NPs). These treatments were compared to assess their effects on plant pigment content.

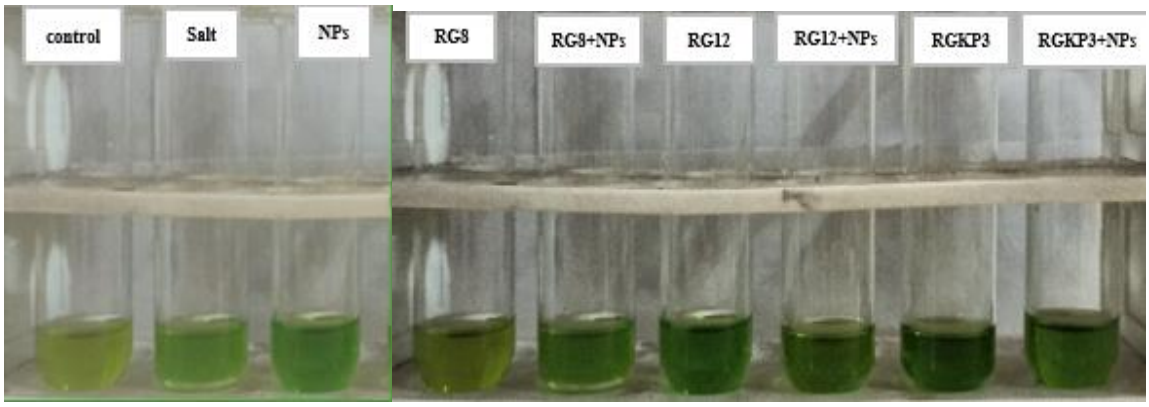

Figure 6.28: Flavonoid estimation was performed for all treated plants under the following conditions: Salt treatment (Zinc acetate, 400 ppm), RG8 (*Pseudomonas songnenensis*), RG8+NPs (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), RG12 (*Bacillus haynesii*), RG12+NPs (*Bacillus haynesii* with 400 ppm ZnO NPs), RGKP3 (*Priestia megaterium*), and RGKP3+NPs (*Priestia megaterium* with 400 ppm ZnO NPs)

Protein Estimation in Plants Treated with Various Treatments: Zinc Acetate (400 ppm), PGPR Isolates (RG8, RG12, RGKP3), and Their Combinations with 400 ppm Zinc Oxide Nanoparticles (RG8+NPs, RG12+NPs, RGKP3+NPs).

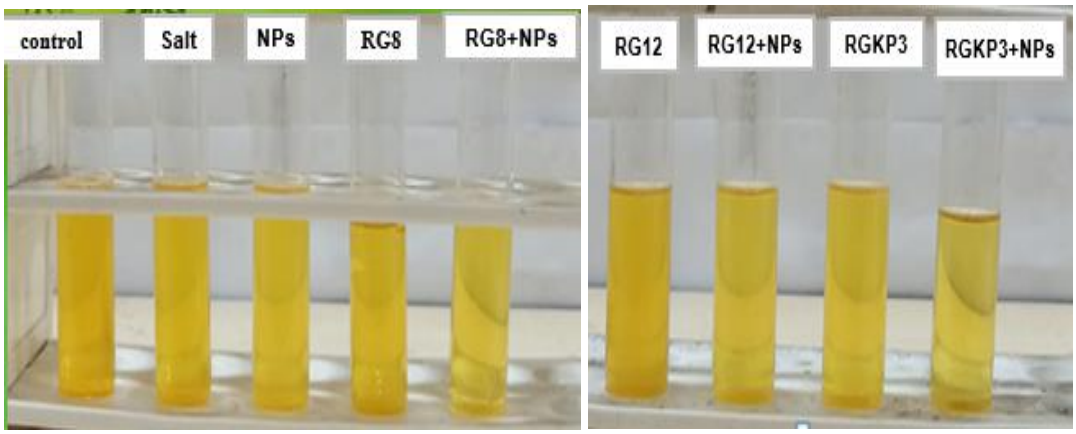

Figure 6.29: Proline estimation was conducted for all treated plants under the following treatments: Salt (Zinc acetate, 400 ppm), RG8 (*Pseudomonas songnenensis*), RG8+NPs (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), RG12 (*Bacillus haynesii*), RG12+NPs (*Bacillus haynesii* with 400 ppm ZnO NPs), RGKP3 (*Priestia megaterium*), and RGKP3+NPs (*Priestia megaterium* with 400 ppm ZnO NPs)

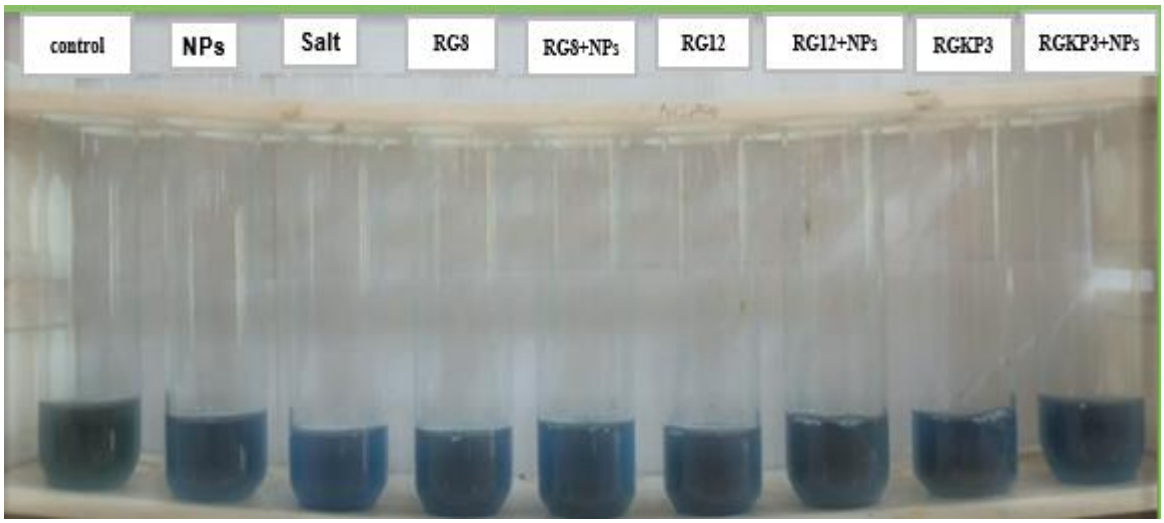

Figure 6.30: Protein estimation was carried out for all treated plants under the following treatments: Salt (Zinc acetate, 400 ppm), RG8 (*Pseudomonas songnenensis*), RG8+NPs (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), RG12 (*Bacillus haynesii*), RG12+NPs (*Bacillus haynesii* with 400 ppm ZnO NPs), RGKP3 (*Priestia megaterium*), and RGKP3+NPs (*Priestia megaterium* with 400 ppm ZnO NPs)

Sugar Estimation in Plants Treated with Various Treatments: Zinc Acetate (400 ppm), PGPR Isolates (RG8, RG12, RGKP3), and Their Combinations with 400 ppm Zinc Oxide Nanoparticles (RG8+NPs, RG12+NPs, RGKP3+NPs).

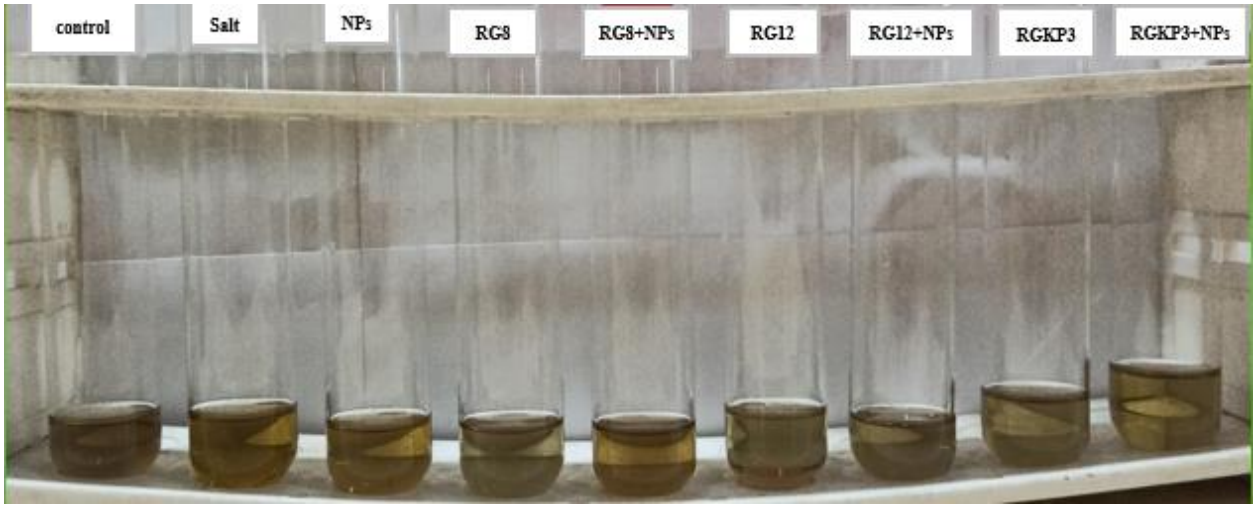

Figure 6.31: Total sugar content was measured for all treated plants under the following treatments: Salt (Zinc acetate, 400 ppm), RG8 (*Pseudomonas songnenensis*), RG8+NPs (*Pseudomonas songnenensis* with 400 ppm ZnO NPs), RG12 (*Bacillus haynesii*), RG12+NPs (*Bacillus haynesii* with 400 ppm ZnO NPs), RGKP3 (*Priestia megaterium*), and RGKP3+NPs (*Priestia megaterium* with 400 ppm ZnO NPs)