CHAPTER: 5

Chemical Synthesis, Characterization, and Optimization Study of ZnO NPs

5.1 Chemically Synthesized ZnO NPs

Zinc oxide nanoparticles ((ZnO NPs) were successfully synthesized using the sol-gel technique, with their formation and dispersion thoroughly characterized. To synthesize the ZnO NPs, 0.02M zinc acetate was dissolved in 100 ml of deionized water and stirred continuously at 180°C for 30-40 minutes. Aqueous 2M sodium hydroxide (NaOH) was then added drop by drop until a white precipitate formed. The mixture was allowed to cool at room temperature for 30 minutes, then placed in an ice bath for 2 hours to allow the particles to settle.

5.2 Characterization of ZnO NPs

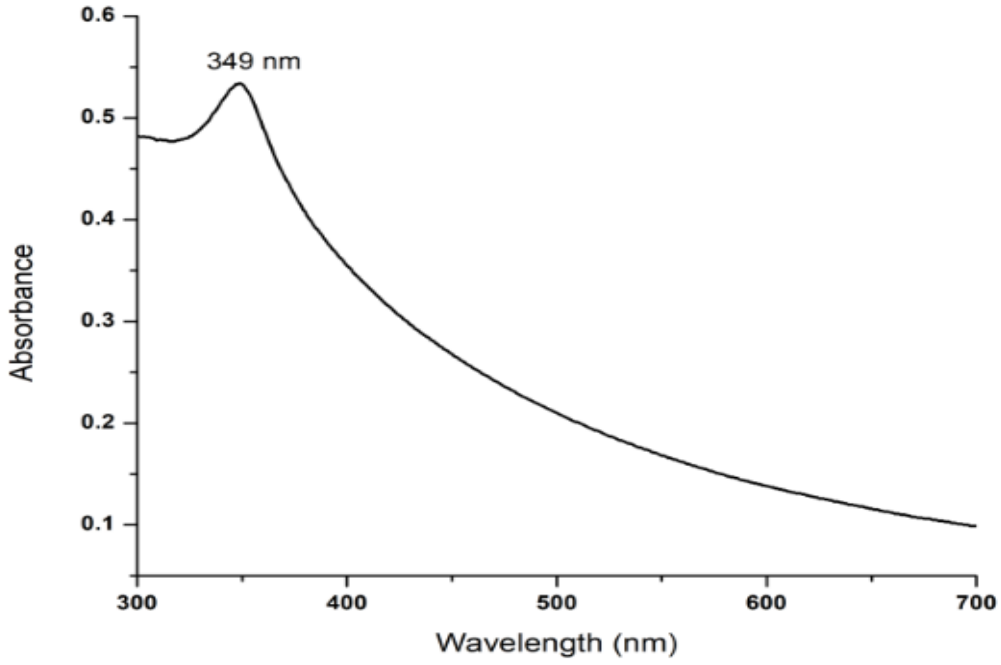

Figure 5.1: UV visible spectrophotometric analysis of ZnO NPs

The solution was centrifuged at 8000 rpm for 10 minutes, and the NPs were analysed using

UV spectrophotometry within the 300–700 nm range. The pellets were dried in a hot air oven overnight at 60°C, during which the zinc hydroxide ($Zn(OH)_2$) solution was converted into ZnO NPs.

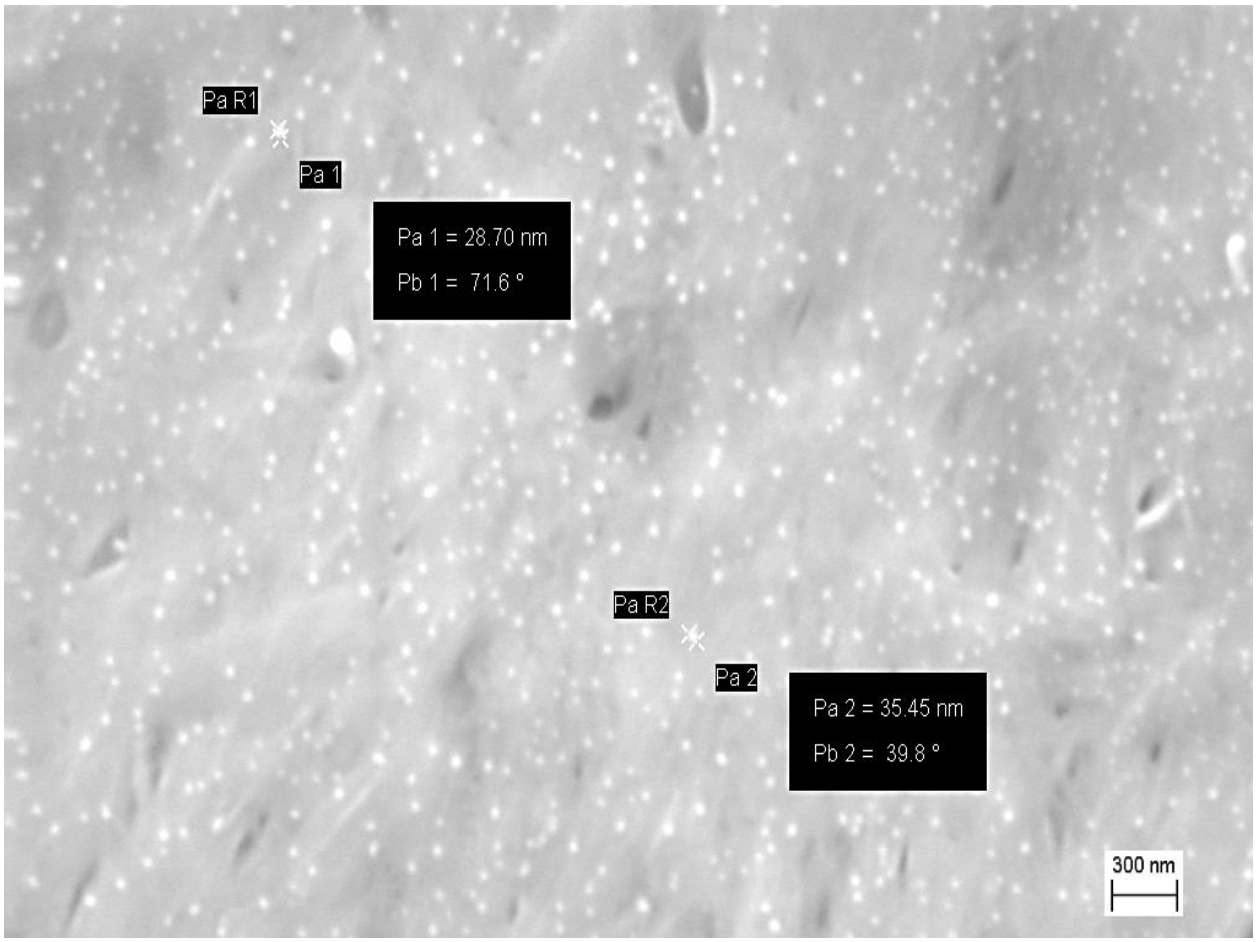

Figure 5.2: SEM micrograph of ZnO NPs (white dots represent nanoparticles)

Scanning Electron Microscopy (SEM) micrographs were employed to examine the size distribution of the nanoparticles that were synthesized. These high-resolution images provided detailed visual insights into the morphology and dimensions of the particles. The analysis revealed that the nanoparticles had a consistent size range, with an average diameter between 30 to 60 nm. This uniformity in size across the sample suggests that the synthesis process was controlled and efficient, producing nanoparticles with consistent nanoscale dimensions. The SEM images thus confirmed the formation of nanoparticles within the desired size range.

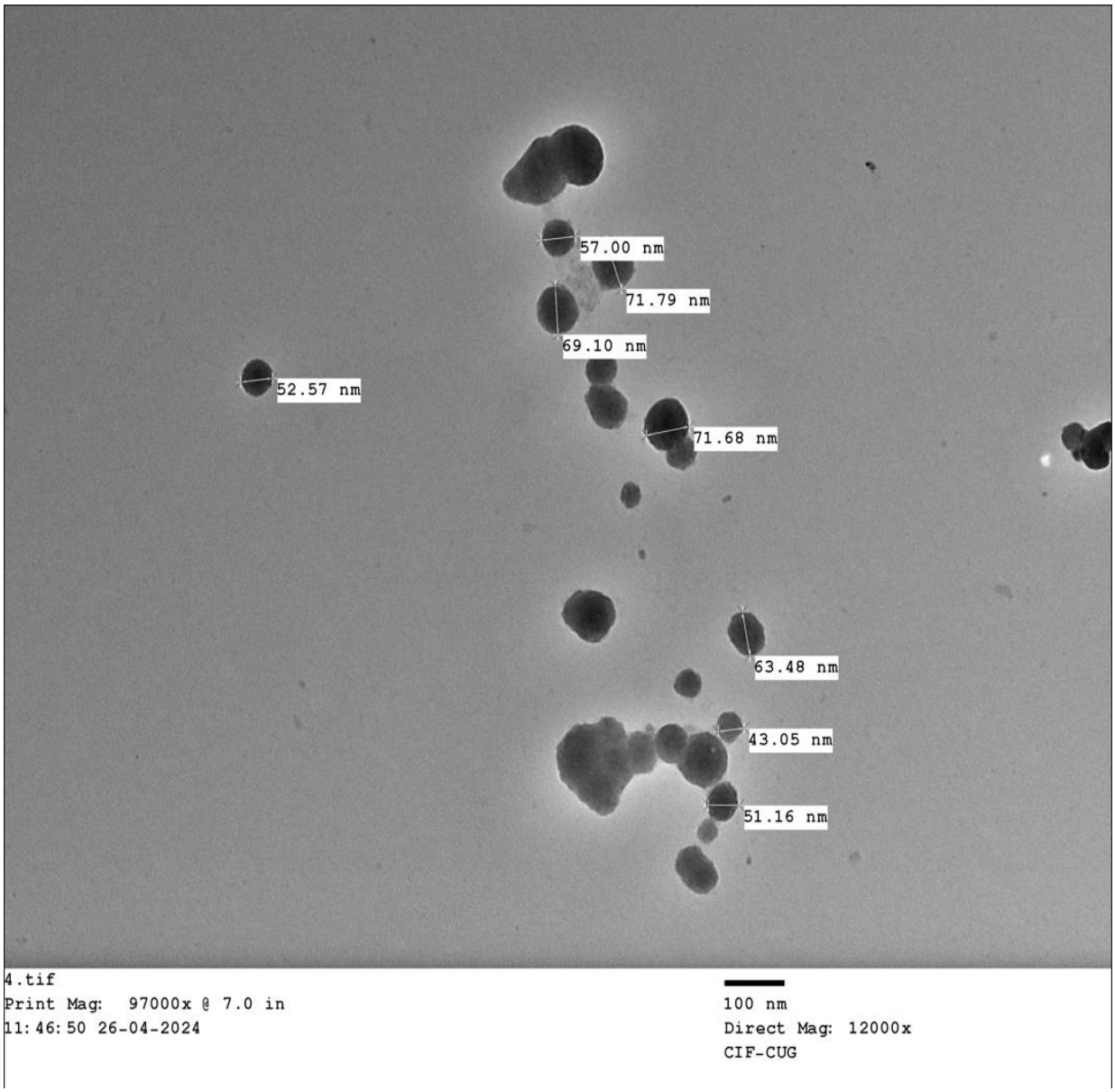

Figure 5.3: TEM micrograph of ZnO NPs with an average diameter of 50-70 nm

Transmission Electron Microscopy (TEM) analysis was conducted to further investigate the size and structure of the zinc oxide nanoparticles (ZnO NPs). The TEM images clearly showed that the nanoparticles had sizes ranging from 43 nm to 72 nm, which confirmed that they fall within the nanoscale range. The scale bar in the images was set at 100 nm, providing a reference point to validate the precise nanoscale dimensions of the ZnO NPs. This detailed analysis emphasized that the nanoparticles maintained their intended size consistency and highlighted their potential for applications that require the high precision and functionality characteristic of nanoscale materials.

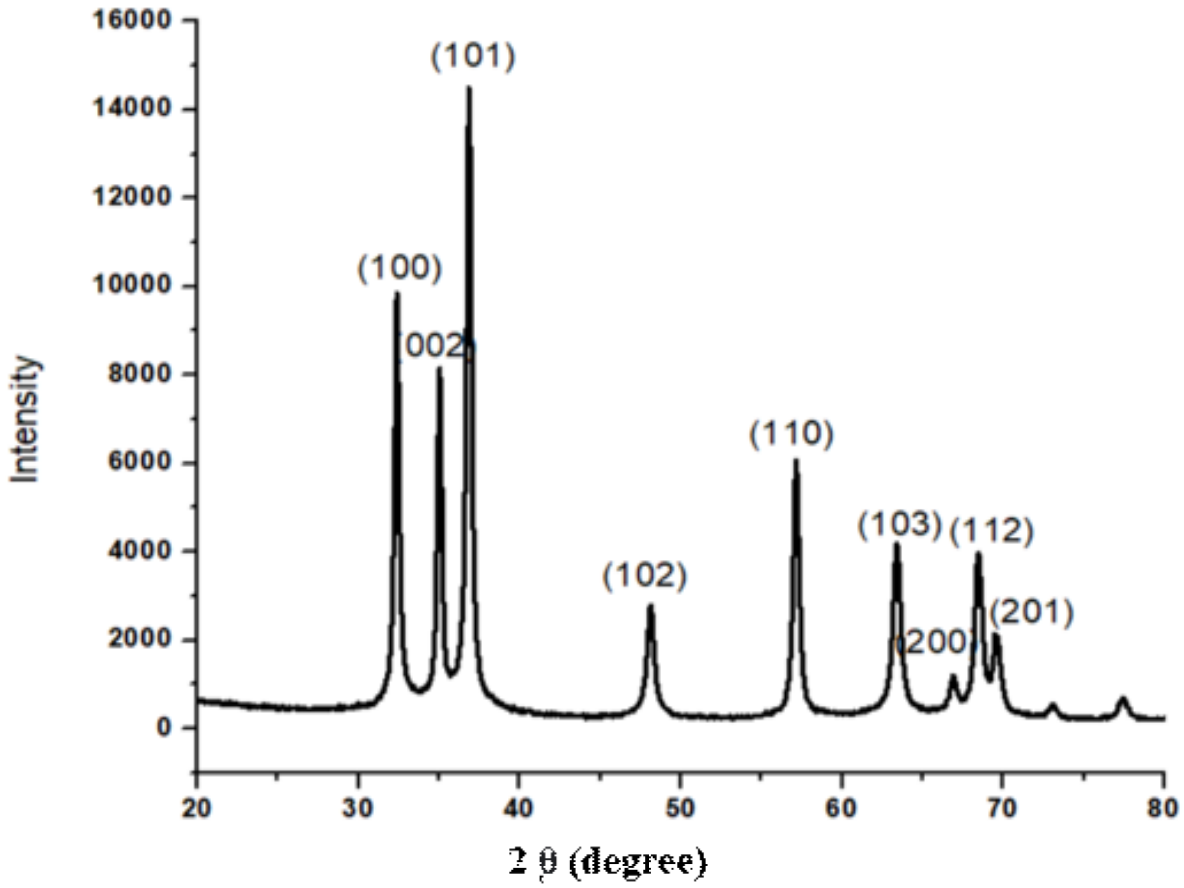

Figure 5.4: X-ray diffraction analysis of chemically synthesized zinc oxide nanoparticles

X-ray diffraction (XRD) analysis was conducted to confirm the structural and phase purity of the synthesized zinc oxide nanoparticles (ZnO NPs). The diffraction patterns obtained were in alignment with the standard reference data from the Joint Committee on Powder Diffraction Standards (JCPDS), specifically card number 36-1451.

5.3 Bacterial growth curve

The bacterial growth curve was analyzed to assess the differential effect of ZnO NPs on the microbial population growth of *Bacillus haynesii*, *Pseudomonas songnenensis*, and *Priestia megaterium*. The population density of these microorganisms initially increased slowly over time, showing positive acceleration, particularly during the lag phase, and followed a typical S-shaped growth curve, characteristic of bacterial growth under favorable conditions.

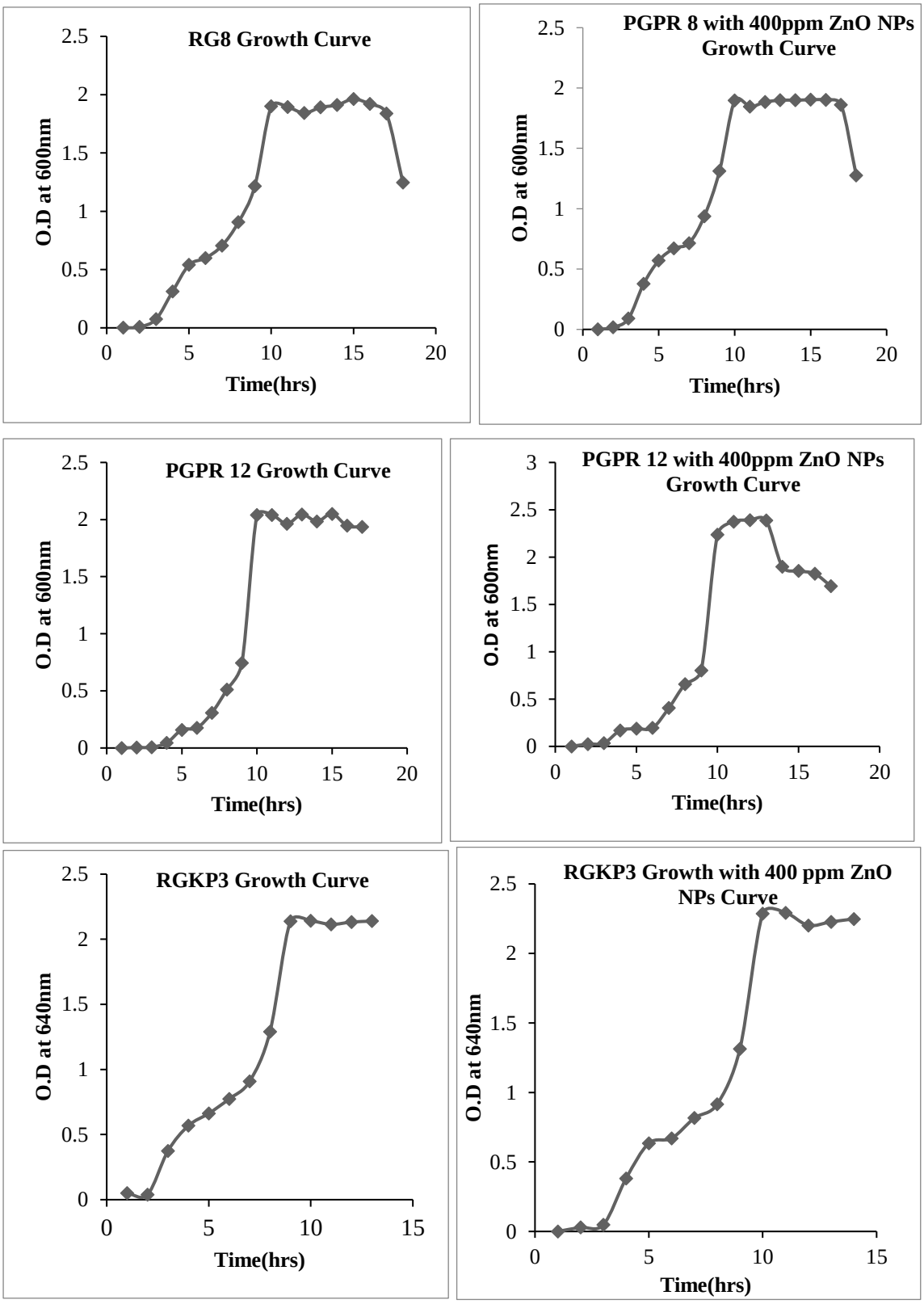

Figure 5.5: Bacterial growth curve of (a) Only RG8 and RG8 with 400 ppm ZnO NPs; (b) Only RG12 and RG12 with 400 ppm ZnO NPs; and (c) Only RGKP3 and RGKP3 with 400 ppm ZnO NPs

Remarkably, the presence of 400 ppm ZnO NPs did not significantly inhibit bacterial growth, with the organisms demonstrating a gradual increase in population density without any signs of slowdown or cessation during the observed period. This suggests that ZnO NPs, at this concentration, do not adversely affect the growth or proliferation of these bacterial species, indicating that the ZnO NPs are likely not toxic at this level. The bacteria maintained their growth course, with steady population density increases during the lag phase, followed by positive acceleration in the exponential phase.

5.4 Seed priming and vigor index

Table 5.1: Effect of Different PGPRs on Seed Germination and Vigor Index

Sr. No.	PGPRs	Seed Germination (%)	Vigor Index
1)	RG8	89%	311.5
2)	RG12	85%	272
3)	RGKP3	92%	349.6

Out of a hundred seeds total of 89% of seeds germinated on the 5th day after inoculation with RG8 and ZnO NPs. A total of 85% of seeds were germinated treated with RG12 + ZnO NPs and 92% of seeds were germinated in RGKP3 with 400 ppm ZnO NPs treated seeds. The seedling lengths of five seeds from RG8 + ZnO NPs, RG12 + ZnO NPs, and RGKP3 ZnO NPs treated were 3.8 cm, 3.2 cm, and 3.5 cm respectively. The vigor index of RG8 with ZnO NPs treated seeds was 311.5, RG12 with ZnO NPs treated seeds was 272 and for RGKP3 with ZnO NPs, the vigor index was 349.6.

Figure 5.6: Groundnut seed Germination with different treatments

5.5 Optimization of the effective concentration of ZnO NPs for plant growth

In this study, the physical parameters of plant growth, such as the number of roots, number of leaves, and root length, were evaluated to assess the impact of PGPR combined with ZnO NPs at a concentration of 400 ppm.

5.5.1 Physical parameters

Figure 5.7: (a) Plants with different treatments in pot experiment after 1 month; Physical parameters of plants with ZnO NPs at various concentration (100 ppm-800 ppm) and untreated.

The results showed that plants treated with both PGPR and ZnO NPs exhibited the maximum number of roots, leaves, and the longest root length, significantly outperforming plants treated with only ZnO NPs.

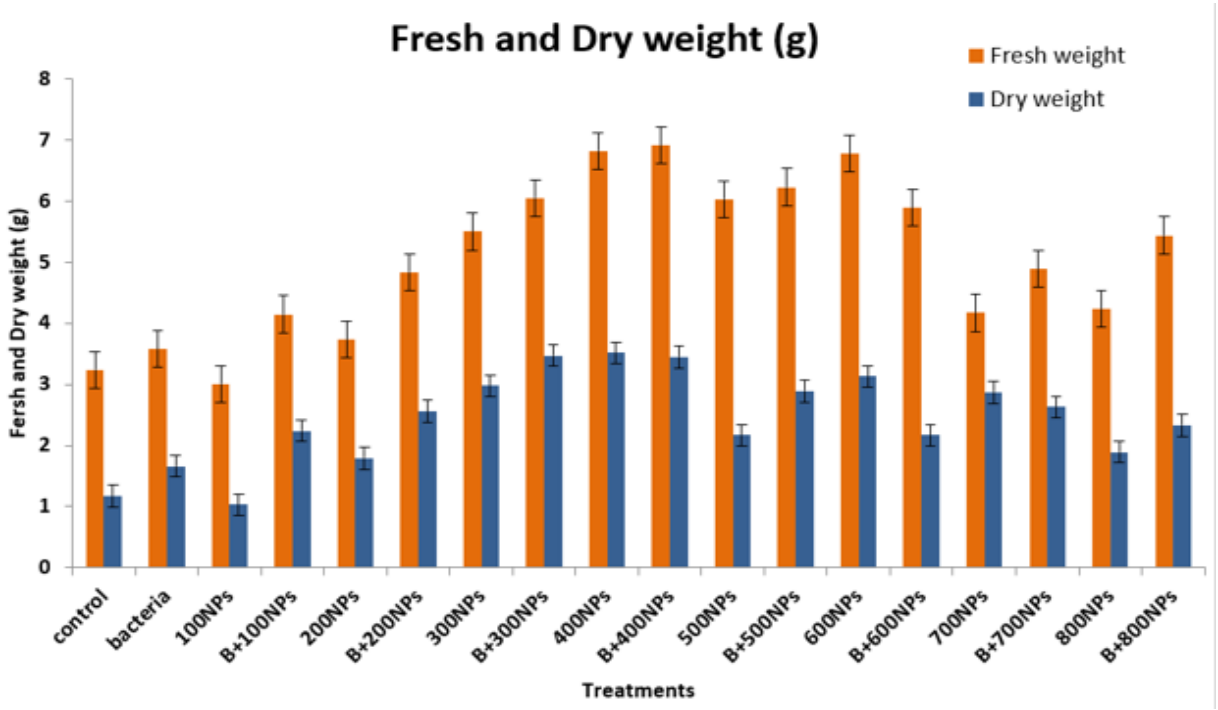

Figure 5.8: Measurements of fresh and dry weight for different treated plants (Bar represents standard error)

The synergistic effect of PGPR and ZnO NPs demonstrated a significantly greater impact on plant growth and development compared to individual treatments with either PGPR or only NPs.

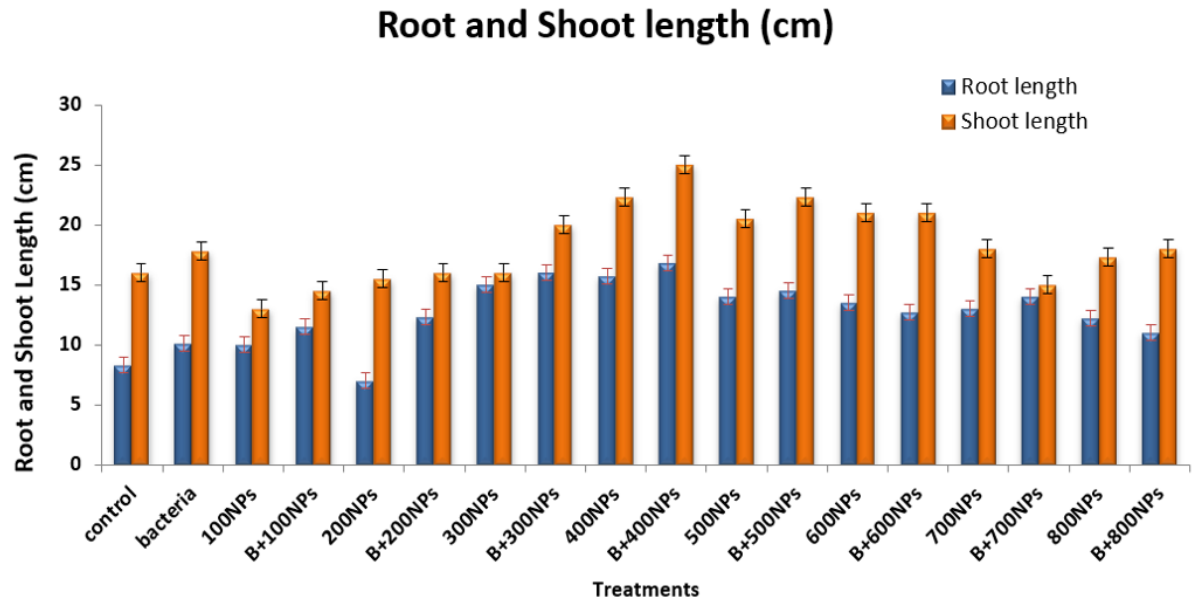

Figure 5.9: Measurements of root and shoot length for different treated plants (Bar represents standard error)

The combined treatment of PGPR isolates and ZnO NPs resulted in substantial improvements in key growth parameters, such as root and shoot length, number of leaves, and overall plant health. When compared to the untreated control plants, those treated with only zinc salt or only ZnO NPs also exhibited improvements in physical parameters, although these changes were not as pronounced as those observed in the combined treatment. This indicates that while both salt and NPs have some positive effects on plant growth, their impact is amplified when used in conjunction with PGPR, suggesting a synergistic relationship that optimizes plant performance.

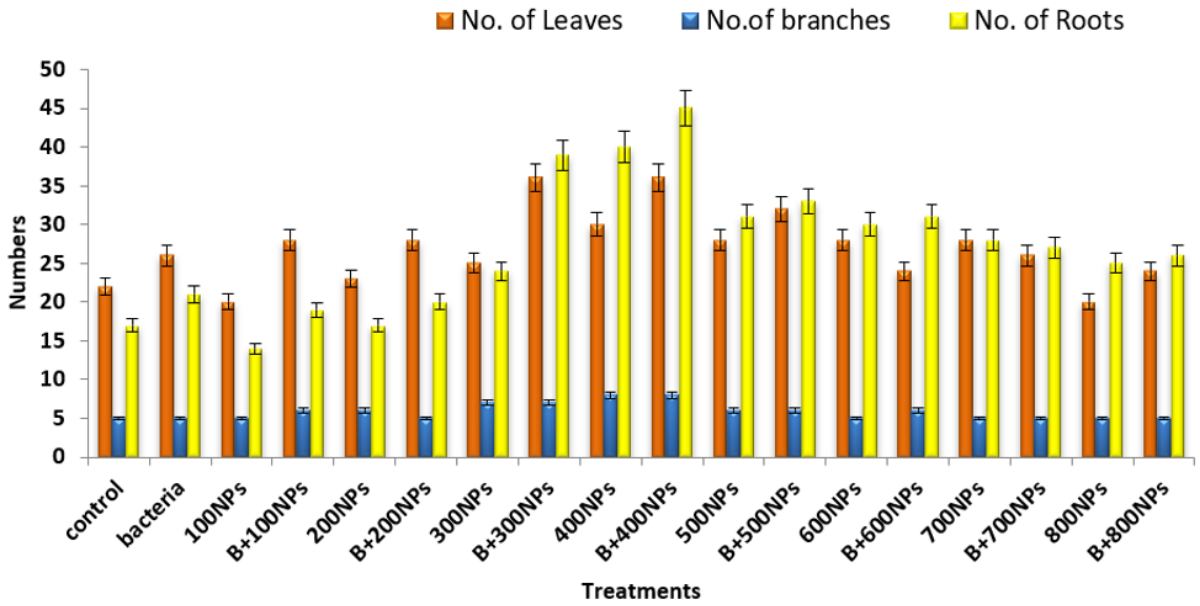

Figure 5.10: Measurements of physical parameters such as Numbers of Leaves, branches, and roots for various concentrations of ZnO NPs in a pot experiment (Bar represents standard error)

5.5.2 Biochemical parameters

Conclusions of the biochemical parameters assessment for all 18 treated plants demonstrated that the production of biochemical parameters in plants is stimulated by the PGPR when combined with an ideal amount of ZnO NPs. Compared to untreated and only NP-treated plants, PGPR and NP-treated plants produced a higher number of biochemical parameters.

A) Total Chlorophyll Content

Chlorophyll a and b, two green plant pigments, are essential cofactors for plants. Chlorophyll a and b play crucial functions in photosynthesis and are also effective singlet oxygen photosensitizers. When compared to other treated plants,

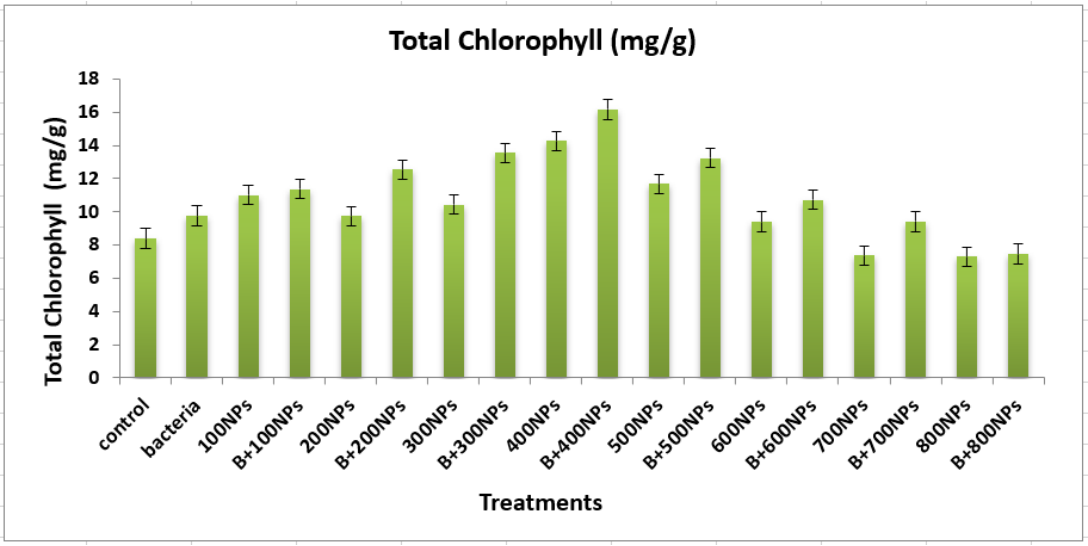

Figure 5.11: Estimation of total chlorophyll content for all different treated plants (Bar represents standard error)

Figure 5.11 showed a significant increase in chlorophyll content in PGPR with 400 ppm ZnO NPs treated plants. The chlorophyll content of the plant decreases from 16.17 mg/g to 7.6 mg/g. Similarly, the zinc oxide concentration decreases from 400 ppm to 100 ppm. AlKahtani et al. (2020) determined that PGPR treatment promoted chlorophyll fluorescence and pigmentation in sweet pepper plants.

B) Carotenoid Content

Carotenoid synthesis in plants can be significantly enhanced in response to stress conditions, primarily due to their vital protective role in photosynthetic regulation. Carotenoids are known to quench excited triplet chlorophyll molecules and singlet oxygen, thereby mitigating the formation of reactive oxygen species (ROS) and protecting the plant from oxidative damage. In this study, an increase in carotenoid content was observed in plant leaves, rising from 27.92 µg/g to 76.64 µg/g, as shown in Fig. 5.12. This increase highlights the plants adaptive response to stress conditions. Furthermore, the role of plant growth-promoting rhizobacteria (PGPRs) in enhancing photosynthetic efficiency is well-

established. PGPRs have been shown to enhance the chlorophyll synthesis pathway with the application of foliar application of Fe-invigorated bacteriosiderophore (Sharma 2017).

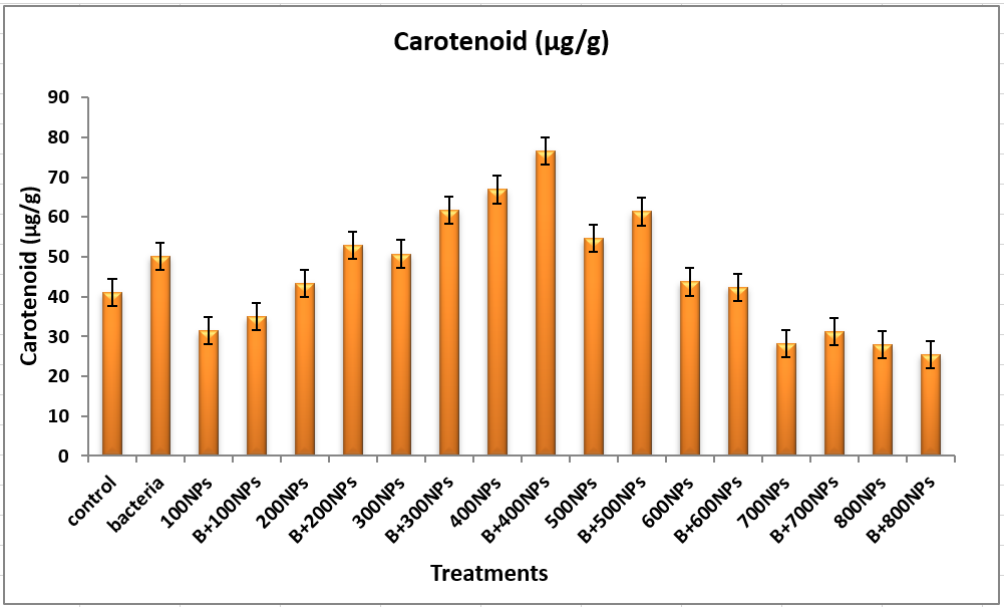

Figure 5.12: Estimation of produced carotenoid content for different treated plants (Bar represents standard error)

C) Flavonoids Content

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

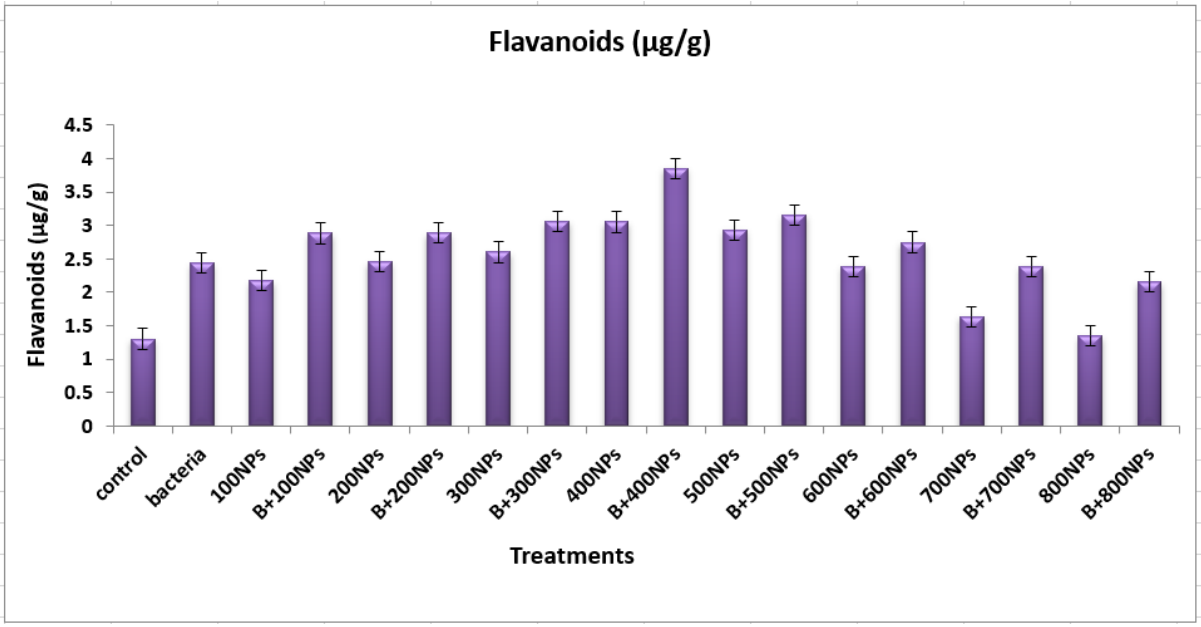

Figure 5.13: Estimation of produced flavonoid content for different treated plants (Bar represents standard error)

Figure 5.13 shows that flavonoids were higher in plants treated with combined RG12 and ZnO NPs compared to other treated plants.

Flavonoid content decreased from 500 ppm to 800 ppm in plants treated with only NPs. In comparison to the control (untreated), only the PGPR treatment also resulted in higher flavonoid content. Nawaz and Bano, (2019) reported maximum flavonoids content in Seeds inoculated with *Pseudomonas stutzeri* and *Pseudomonas putida* with Ag NPs over untreated plants on cucumber plant.

D) Proline Content

Proline, an amino acid, is essential in plants. It protects plants from various stressors and also aids plant recovery from stress. The proline content was reduced in all treatments of PGPR and along with NPs + PGPR. The maximum increase in proline content was 52.8% compared to treatment with PGPR+NPs. The proline content of plant leaves increased from 14.112 mg/g to 38.612 mg/g. However, both the single and combination application of PGPR and ZnO NPs at 100 ppm to 800 ppm concentrations demonstrated a decrease in proline content when compared to the 200 ppm and 800 ppm ZnO NP concentrations.

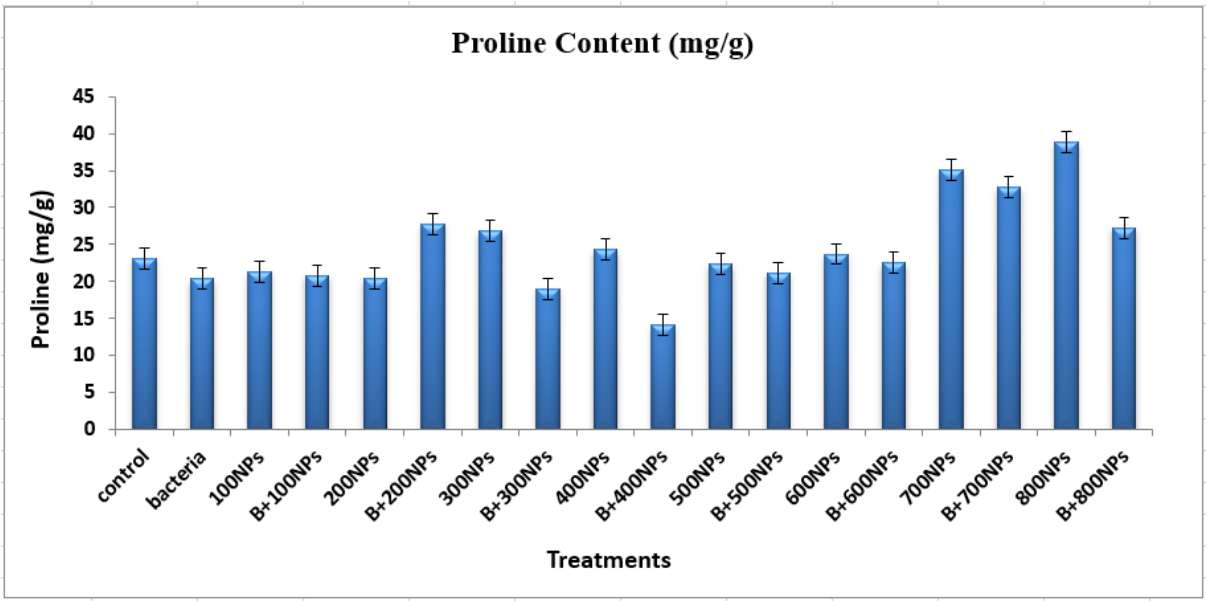

Figure 5.14: Estimation of produced proline content for different treated plants (Bar represents standard error)

The treatment with PGPR and ZnO NPs at 400 ppm resulted in the least quantity of proline content. Figure 5.14 revealed that the maximum decrease was 14.112 mg/g in PGPR with

400 ppm ZnO NPs which was 3.26% less than that of the control (untreated) plant. Isfahani et al., (2019) reported that the results indicated a significant increase in the proline content associated with increasing the stress level. The proline content was significantly decreased by the use of silicon nanoparticles with *Citrobacter freundii* in tomato plant.

E) Total Protein Content

Plant proteins have a variety of enzymatic, structural, and functional activities. They also serve as storage media for the growing development and nutritional needs of the seedling.

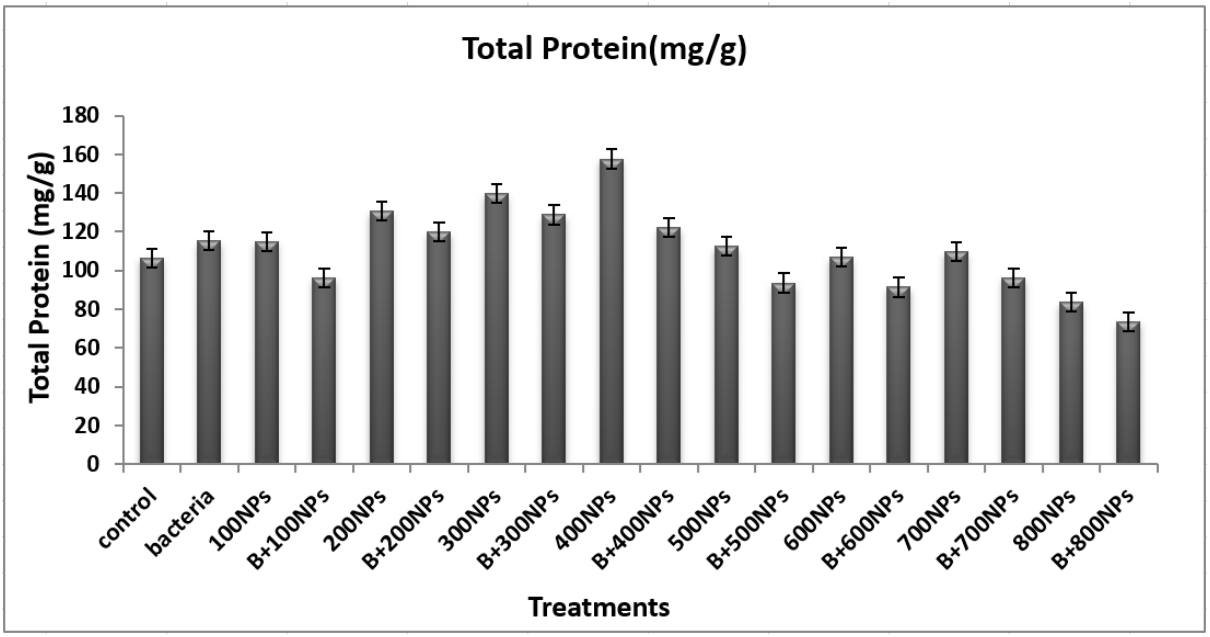

Figure 5.15: Estimation of produced protein content for different treated plants (Bar represents standard error)

Figure 5.15 denoted that the total protein in plant leaves increased as the concentration of ZnO NPs increased. Different concentrations of ZnO NPs produced different protein amounts. Only PGPR-treated plants produced total protein is higher than untreated plants. PGPR at 400 ppm concentration of ZnO NPs produced a maximum protein which is 89% more compared to control (untreated).

F) Sugar Content

Sugars have an impact on all stages of the plant life cycle, interact with other signaling molecules such as phytohormones, and regulate plant growth and development. The greatest amount of total sugar (66.066 mg/g) was obtained by applying combined RG12+400 ppm ZnO NPs (Fig 5.16). According to Stefan et al. (2013), the PGPR strains increase the total reducing carbohydrate (sugar) content up to 49.28%, which promotes the

nutritional value of the harvested runner bean grains.

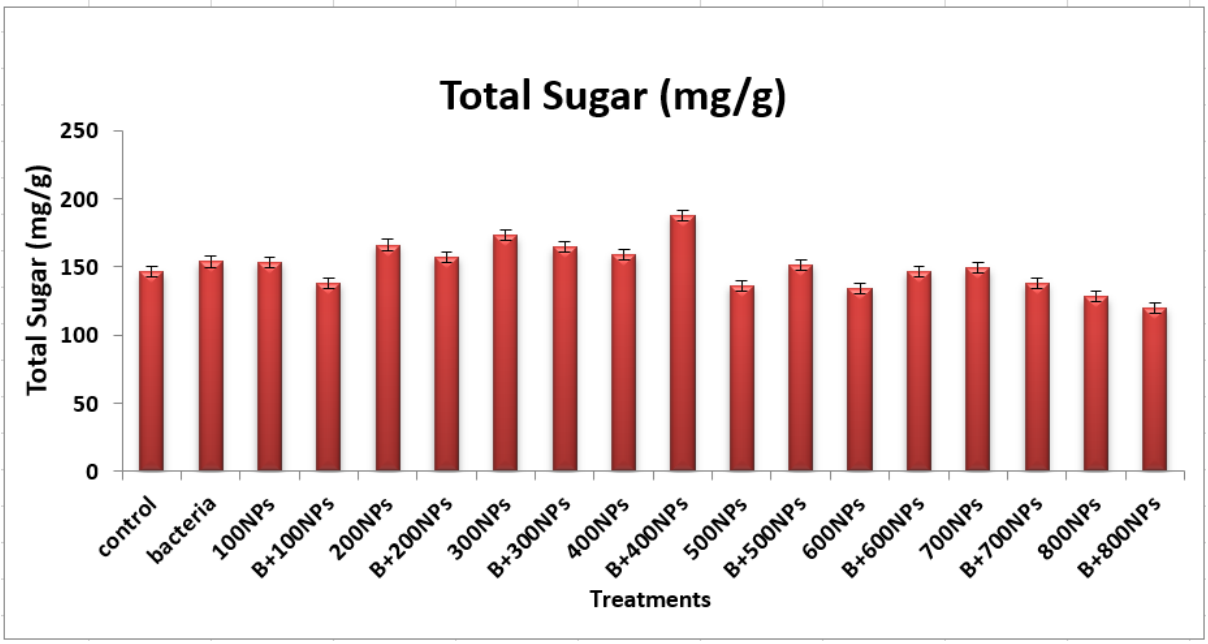

Figure 5.16: Estimation of produced total sugar content for different treated plants (Bar represents standard error)

5.7 Effect of PGPR (RG12) and ZnO NPs (400 ppm) on plant growth and development

PGPR strain *Bacillus haynesii* combined with synthesized ZnO NPs at 400 ppm for assessment of the groundnut plants growth and development. RG12 with a previously optimized NP value helps to improve physicochemical metrics as well as produce a higher amount of biochemical content as compared to untreated plants.