CHAPTER:8

SUMMARY

Modern agriculture faces a range of significant challenges, including declining soil fertility, and increasing dependence on chemical fertilizers. These problems make it difficult for farmers to maintain high crop yields while also protecting the environment. There is an urgent need for sustainable solutions that can boost agricultural productivity, reduce the harmful impact of chemical inputs, and improve soil health. This research addresses these challenges by focusing on two promising solutions: PGPR and MONPs. PGPR are beneficial bacteria that naturally support plant growth by improving soil health, enhancing nutrient cycling, fixing nitrogen, and helping plants to tolerate stress. On the other hand, MONPs, especially ZnO NPs, have shown a potential to improve nutrient availability to plants, helping them grow stronger and more resilient. Despite their benefits, there are concerns about the environmental toxicity of MONPs, particularly if they are used in high concentrations.

By combining biotechnology (PGPR) with nanotechnology (MONPs), this study offers a promising approach to overcoming the challenges faced by modern agriculture. It helps reduce the reliance on harmful chemical inputs, improves soil health, and increases crop productivity. Furthermore, this research proposes a more sustainable and eco-friendly way to protect crops from environmental stresses, contributing to agricultural resilience in the face of climate change and resource shortages. Overall, the integration of PGPR and MONPs presents a balanced solution that can improve agricultural productivity, reduce environmental damage, and support long-term sustainability in farming.

This study investigates the effects of combining potent PGPR strains (RG8, RG12, and RGKP3) with ZnO NPs on the growth and productivity of groundnut plants in controlled pot experiments. By integrating biological and nanotechnological advancements, this research contributes to sustainable agricultural practices, emphasizing the importance of innovative strategies to ensure food security and environmental sustainability.

The integration of nanomaterials with PGPR offers significant potential for advancing agricultural productivity. This study investigated the co-application of ZnO NPs and PGPR to enhance the growth of groundnut plants. The research evaluated plant growth under

treatments involving NPs and PGPR both individually and in combination to determine their effects on plant development. The study aimed to achieve several objectives: first, isolating bacteria from the selected soil and screening these isolates for various plant growth-promoting traits; second, identifying the selected bacterial isolates through molecular characterization using the 16S rRNA technique; third, optimizing the synthesis and concentration of NPs for effective plant growth enhancement; and then, conducting a pot experiment to assess the plant growth-promoting potential of the selected PGPR strains individually and in combination with NPs. This approach aimed to provide insights into the cumulative effects of these innovative agricultural tools for sustainable crop production.

The research methodology was designed to evaluate the co-application of PGPR and ZnO NPs for enhancing groundnut plant growth. Soil samples were collected from groundnut fields in Saurashtra, Gujarat, to isolate PGPR. These soil samples were screened for potent PGPR strains exhibiting key traits associated with plant growth promotion. Three promising strains were selected for further analysis: *Pseudomonas songnenensis* (RG8), *Bacillus haynesii* (RG12), and *Priestia megaterium* (RGKP3). Molecular identification of these strains was performed using 16S rRNA sequencing to confirm their species.

ZnO NPs were synthesized chemically using sol-gel method. The concentration of the synthesized NPs was optimized at 400 ppm to ensure their efficacy and safety. Characterization of ZnO NPs was conducted using UV-visible spectrophotometry, X-ray diffraction (XRD), scanning electron microscopy, and High-Resolution Transmission Electron Microscopy (HR-TEM) to confirm their size, structure, and properties. Optimization studies were conducted to assess the compatibility of the various concentrations (100-800 ppm) of ZnO NPs with groundnut plants, ensuring its safe application.

A pot experiment was carried out to evaluate the combined effect of the selected PGPR strains and the optimized ZnO NPs concentration on the growth of groundnut plants. The growth parameters evaluated included the seed germination rate, root and shoot growth, along with a range of biochemical traits. The chlorophyll content was determined using the Arnon method (Arnon, 1949), which involves measuring OD at three distinct wavelengths: 645 nm for chlorophyll a, 663 nm for chlorophyll b, and 480 nm for carotenoids. Additionally, the flavonoid content was quantified following the method of Zhishen et al.

(1999), with absorbance measured at 430 nm against a blank. Proline content in the leaves was assessed using the method outlined by Bates et al. (1973). Furthermore, reducing sugars were quantified using the Dubois method (Dubois et al., 1956), a colorimetric assay that forms a colored complex with phenol and sulfuric acid. Moreover, the protein content of the leaves was measured using Bradford's method (Bradford, 1976), with a standard curve generated using Bovine Serum Albumin (BSA) in the concentration range of 20-100 mg/g. This comprehensive analysis provided insights into the combined effects of PGPR and ZnO NPs on enhancing plant growth and biochemical parameters.

The study examined the characteristics of 84 isolates, with notable findings on their plant growth-promoting activities. Thirty-five isolates produced indole-3-acetic acid (IAA) ranging from 20.7 to 133 µg/mL, with RGKP3 showing the highest production. RG5 produced the maximum amount of ammonia (55.5 µg/mL), which could enhance soil nitrogen. HCN production, observed in 26 isolates, indicated potential for bioremediation and pathogen antagonism. Four isolates, including RG12, demonstrated phosphate solubilization, with RG12 achieving the highest solubilization (205 µg/mL on day 7). Gibberellin production ranged from 10.2 to 112.16 µg/mL in six isolates, with RGK7 as the highest giberellin producer. Seven isolates exhibited chitinase activity, and four isolates, including RGKP3, produced siderophores. Potassium solubilization was observed in all isolates except RGKP7, with RGKP4 forming the largest halo zone (4.5 cm). Nitrogen fixation was present in 22 isolates, and six isolates solubilized zinc, vital for enzymatic processes. These findings highlight the diverse and beneficial traits of the isolates, indicating their potential for enhancing soil health and supporting plant growth.

Gram staining identified RG8 as Gram-negative, while RG12 and RGKP3 were Gram-positive. Phylogenetic analysis through 16S rRNA sequencing confirmed the identities of RG8 (*Pseudomonas songnenensis*), RG12 (*Bacillus haynesii*), and RGKP3 (*Priestia megaterium*), with accession numbers OP445813, OP445814, and OP528743, respectively, submitted to GenBank.

ZnO NPs synthesized using the sol-gel technique showed a concentration of 400 ppm. Characterization by UV-Vis spectrophotometry, SEM, HR-TEM, and XRD confirmed their well-dispersed nanoscale morphology. Furthermore, growth curves of *Bacillus haynesii*,

Pseudomonas songnenensis, and *Priestia megaterium* indicated no inhibitory effects from 400 ppm ZnO NPs.

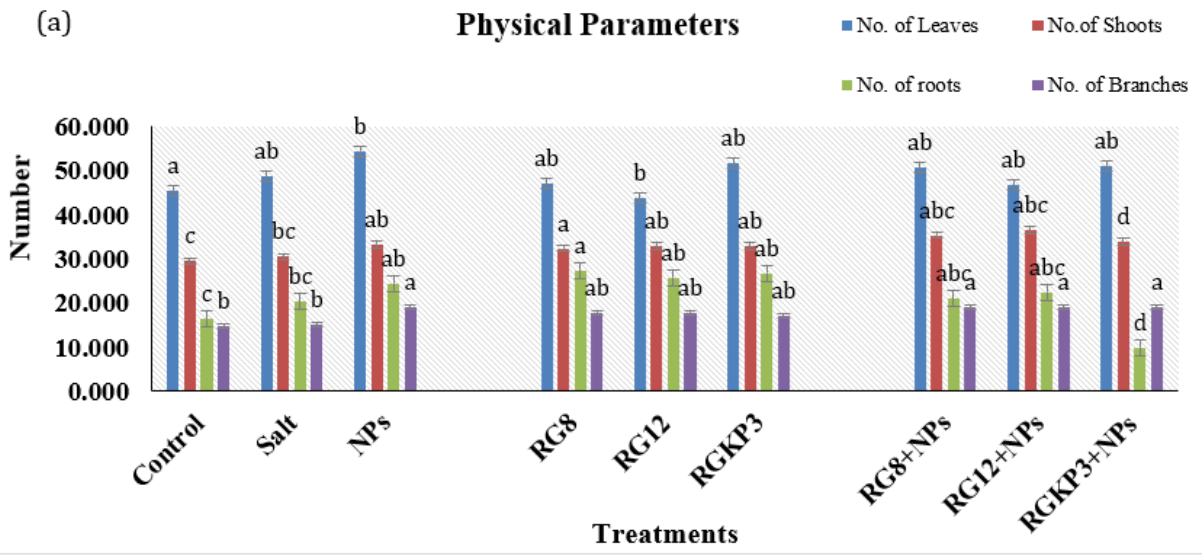

Figure 8.1: Physical parameters such as No. of roots, No. of branch, No. of leaves, No. of shoots h of Groundnut Plants with different treatments in pot experiment after 1 month of in vitro experiment with combined application of three potent PGPR (RG8, RG12 and RGKP3) strain with ZnO NPs. focusing on 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

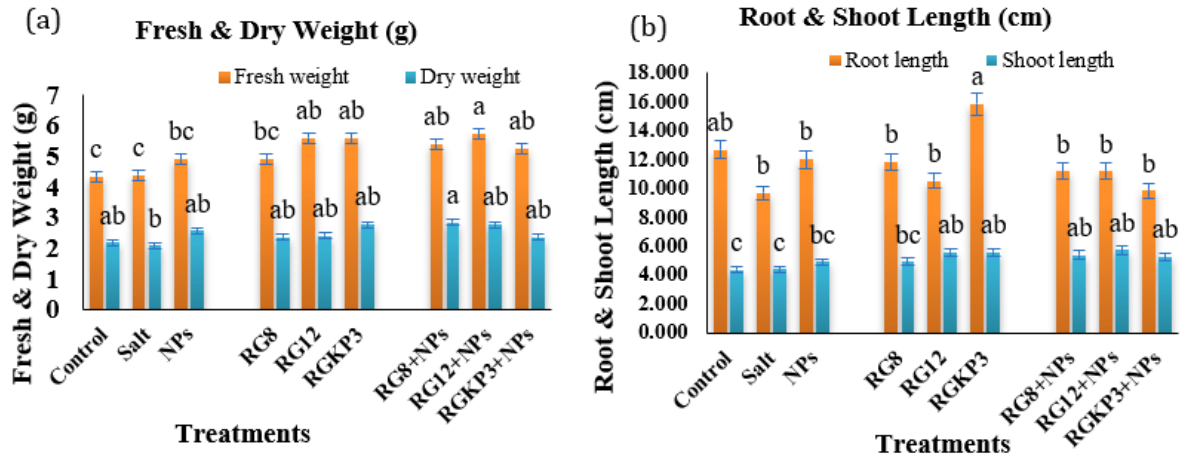

Figure 8.2: Physical parameters such as a) Fresh and Dry weights and b) Root and shoot length of Groundnut Plants with different treatments in pot experiment after 1 month of in vitro experiment with combined application of three potent PGPR (RG8, RG12 and RGKP3) strain with ZnO NPs. focusing on SSR 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

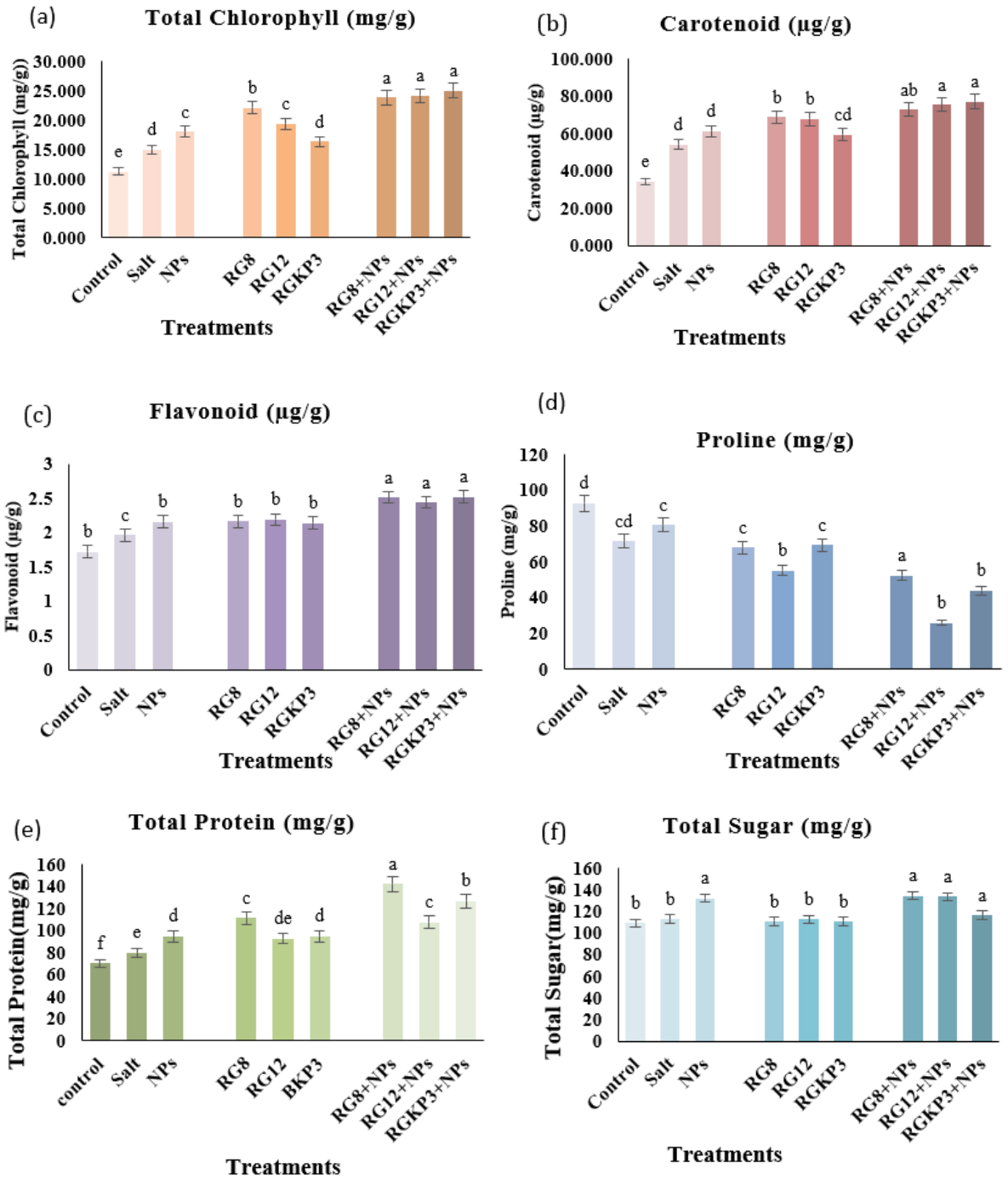

Figure 8.3: Estimation of (a) total chlorophyll content; (b) carotenoids content; (c) flavonoids content; (d) proline content for differently treated plants; (e) produced total protein content; and (f) total sugar content in plants with a combination of PGPRs RG8, RG12 and RGKP3 and ZnO NPs in a pot 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

Treatment with 400 ppm ZnO NPs significantly improved seed germination and vigor index, resulting in germination rates of 89%, 85%, and 92% for RG8, RG12, and RGKP3, respectively. Seedling lengths and vigor indices also improved, with RGKP3 showing the highest vigor index (349.6). Optimization of ZnO NPs concentration revealed that combined treatments (PGPR + ZnO NPs at 400 ppm) maximized physical parameters such as root and leaf growth while improving biochemical parameters like chlorophyll, carotenoids, flavonoids, sugar, and protein content.

Combined PGPR and ZnO NP application enhanced chlorophyll content, reaching 24.80 mg/g with RGKP3 + ZnO NPs. Carotenoid levels were maximum at 77.4 µg/g, and flavonoid content increased by 68.5% over controls. Proline content, a stress marker, was lowest in RG12 + ZnO NP treatments. Sugar and protein levels were notably higher, with RG8 + ZnO NPs yielding the highest protein content (134.19 mg/g), doubling that of untreated plants. These results affirm the efficacy of combining PGPR and ZnO NPs in promoting sustainable agricultural practices by enhancing plant growth and biochemical attributes, particularly with RGKP3 proving to be the most effective strain.