

COMPARATIVE ANALYSIS OF FOLIAR APPLICATION OF ELEMENTAL SELENIUM AND SE-NANOPARTICLES ON PLANT GROWTH

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Abstract

Background: Selenium (Se) is a vital trace micronutrient whose deficiency poses a significant global population health concern due to its unequal distribution in agricultural lands as well as insufficient bioavailability in plant foods.

Objective: This study aimed to isolate and characterize selenium-resistant plant growth-promoting bacteria from agricultural soil and biosynthesize selenium nanoparticles. It comparatively evaluated the effects of Se-NP foliar spray application on growth, biomass, protein content, and chlorophyll pigments in *Lactuca sativa* under greenhouse conditions.

Methods: Fifteen bacterial isolates were screened for selenite reduction; five Se-reducing strains (A1-SER–A5-SER) were selected and characterized morphologically and biochemically including heavy-metal resistance, and auxin production. Biogenic Se-NPs were tested as a foliar spray in a 6–7-week greenhouse trial against bacterial inoculation and elemental selenium assessing fresh weight, soluble protein, and photosynthetic pigments.

Results: All strains were Gram-positive, spore-forming rods, catalase-positive, selenite-tolerant, and grew optimally at 28°C / pH 7 with resistance to Nickel, Zinc, and Manganese, and high levels of auxin production. Se-NP foliar spray outperformed other treatments increasing biomass (18.6 g) and chlorophyll A content (4.901 µg/g fresh weight).

Conclusion: Biogenic selenium nanoparticles synthesized from locally isolated selenium-resistant bacteria, applied as a foliar spray, proved most effective for selenium biofortification and plant growth promotion among the treatments tested. This approach offers a cost-effective, sustainable strategy for micronutrient management and lays a foundation for microbial nanotechnology applications in addressing crop micronutrient deficiency.

Introduction

Micronutrient deficiency represents one of the most persistent and unaddressed threats to global public health, affecting billions of people across both developing and developed nations (Bailey, West Jr, & Black, 2015). Selenium is an essential trace element playing a key role in ensuring the health of human beings, mostly through its integration in seleno-proteins, which

are linked to defense against oxidative stress, immune regulation, and cellular redox balance (Papp, Lu, Holmgren, & Khanna, 2007). Sufficient selenium consumption has been linked to fewer risks of cardiovascular diseases, cancer, and oxidative stress-related diseases (Barchielli, Capperucci, & Tanini, 2022). Low selenium intake has further been associated with elevated risk of several cancers including

prostate, lung, and esophageal malignancies, as well as cardiovascular disease and oxidative stress-related conditions, whereas adequate selenium status has been shown to reduce cancer risk, enhance immune response, and protect against viral infections and cellular damage (Rayman, 2012). Regardless of its significance, selenium deficiency has been a nutritional problem in the global scenario because the degree of distribution of selenium in soil directly translates to the level of selenium in crops and, hence, the human diet (Fordyce, 2012). This element is also found in inorganic form in soil systems in agricultural systems, such as selenite and selenate (SeO_4^{2-}) which is absorbed by plants and transformed into organic forms including selenomethionine and selenocysteine, which are considerably more bioavailable to humans than their inorganic counterparts (Mondal, Biswas, Bordoloi, Santra, & Majumdar, 2025).

Nevertheless, bioavailability of selenium in soil varies due to various variables such as pH, the content of organic matters and redox conditions, as a consequence, selenium concentrations in plant-derived food vary enormously worldwide, with soils across large parts of Asia, Africa, and Europe documented as severely selenium-deficient (Celletti & Schiavon, 2026). However, at very high concentrations, selenium may cause toxicity in plants, causing disturbances in sulfur metabolism, oxidative stress, problems in seed germination and development of crops (Gupta & Gupta, 2017). Therefore, the concentration, chemical form and mode of application of selenium are important factors in determining its effect on plants and its potential for agricultural use.

Biofortification is the process by which the concentration of certain micronutrients in food crops is increased via agronomical or biological methods during plant growth (Ofori, Antonello, English, & Aryee, 2022). It has been suggested as a feasible means for enhancing nutritional value of crops and alleviating deficiencies of micronutrients (Avnee, Sood, Chaudhary, Jhorar, & Rana, 2023). Several methods have been tested for selenium biofortification among which foliar application is highly promising as in this technique,

selenium is applied to the aerial parts of plants, thereby limiting the effect of soil parameters on its effectiveness (Galić, Vinković, Ravnjak, & Lončarić, 2021). The effectiveness of foliar selenium application has been evaluated in various crops such as rice, wheat, lettuce, spinach and buckwheat (Oztekin & Buyuktuncer, 2025).

The transformation of selenium is also an important function of soil microorganisms. Certain bacterial species can convert soluble selenite and selenate to insoluble, less toxic elemental selenium (Se^0), through dissimilatory reduction pathways (Eswayah, Smith, & Gardiner, 2016). In addition to the microbial reduction process, Se-NPs formation is also observed, which helps in the microbial synthesis of Se-NPs (Zambonino *et al.*, 2021). In contrast to the conventional physiochemical techniques, microbial synthesis of Se-NPs can be performed at relatively mild conditions and can also minimize the consumption of chemical reagents (Qin, Wang, Lai, Liang, & Qian, 2025). *Bacillus* species have been repeatedly identified as particularly efficient selenium reducers, combining robust environmental stress tolerance, heavy metal resistance, and nanoparticle synthesis capacity with additional plant growth-promoting traits such as auxin biosynthesis and phosphate solubilization (Arshad *et al.*, 2026). The above properties indicate that these strains of *Bacillus* are promising producers of Se-NPs and can be used in agriculture. The increasing interest in biogenic Se-NPs stems from their nanoscale properties that can impact the bioavailability of selenium and its interactions with plant tissues (Mahakalkar *et al.*, 2026). This may be an alternative way to supply selenium to plants when using the foliar spray application. However, the effects of selenium depend on its chemical form and mode of delivery. The comparison of the effects of bacterial inoculation, elemental selenium, and biogenic selenium nanoparticles under identical experimental conditions may shed more light on the impact of these forms on plant physiology.

Lactuca sativa (lettuce) was selected as the plant to be used in this study due to its nutritional significance, popularity, and short growth cycle (Hong *et al.*, 2022). Despite the emerging

interest in selenium biofortification and nanoparticle synthesis by microorganisms, there are few studies in the literature comparing the impact of elemental selenium, bacterial inoculation, and Se-NP foliar application in the same plant under greenhouse conditions (Burmistrov, Shumeyko, Semenova, Dorokhov, & Gudkov, 2025). Most of the research in the literature either deals with the chemical method for the use of selenium or uses microbes but not comparing both techniques with biologically synthesized nanoparticles by bacteria from the local soil (Sorongpong, Das, Das, Chakraborty, & Pandey, 2026). Another study also screened selenium resistant and Plant growth promoting bacteria from agricultural soils (Galić, Vinković, Ravnjak, & Lončarić, 2021). Se-NPs were then biosynthesized using selected bacterial isolates. A greenhouse experiment using *L. sativa* was conducted to comparatively evaluate bacterial inoculation, elemental selenium application, and biogenic Se-NP foliar treatment (Shang *et al.*, 2023). The effects of these treatments were evaluated based on plant fresh biomass, soluble protein content, and photosynthetic pigments (Kučerová, Henselová, Slováková, & Hensel, 2019). The study aimed to assess the potential of selenium-reducing bacterial isolates and their biogenic Se-NPs as biological approaches for improving plant performance and supporting selenium biofortification (Sonali, Gayathri, Rangasamy, Kumar, & Rajagopal, 2024).

2. Materials and Methods

2.1 Sample Collection and Isolation of Selenium-Resistant Bacteria

Three soil samples were taken and used for the isolation of Selenium resistant bacteria. The samples were serially diluted and then seeded in nutrient agar plates containing 800µl of sodium selenite (Na_2SeO_3) to enrich for selenite reducing organisms (Shahdin, Batool, Shafi, & Siddiq, 2024). Plates were incubated at 37°C for 24h, and colonies exhibiting a distinct red coloration indicative of active reduction of selenite to elemental selenium were selected for further study (Dwivedi, AlKhedhairy, Ahamed, & Musarrat, 2013). Selected colonies were repeatedly streaked onto fresh nutrient agar and re-incubated at 37°C for 24 h for purification. From the fifteen isolates tested, five strains that

exhibited regular selenite reduction and strong growth were retained and named A1-SER, A2-SER, A3-SER, A4-SER and A5-SER.

2.2 Morphological Characterization

Colony morphology of the five pure cultures was observed after 24 h of incubation on nutrient agar at 37°C based on their color, form, size, elevation, margin, and surface appearance (Sehair, Ahmed, & Khalid, 2025). Cell morphology and gram reactions were determined by gram staining of 24 h old cultures. Heat-fixed smears were stained with crystal violet, treated with iodine as a mordant to form the crystal violet-iodine complex, and decolorized with ethanol before counter staining with safranin for 45s (Patra, Das, Das, & Thatoi, 2020). Gram-positive cells retained the primary violet stain owing to their thick peptidoglycan layer, while Gram-negative cells were decolorized and appeared pink after counter staining (Moyes, Reynolds, & Breakwell, 2009). Endospore formation was assessed after 7 days of growth using the Schaeffer-Fulton method. Heat-fixed smears were flooded with malachite green and steamed over boiling water for approximately 5 min to drive the stain into any endospores then cooled, rinsed, and counter stained with safranin for 45 s (Oktari, 2017). Slides were air-dried and examined under oil-immersion microscopy.

2.3 Biochemical Characterization

Catalase test. A loopful 24 hours old culture was smeared on a slide and covered with 3% hydrogen peroxide. Formation of bubbles immediately is a positive reaction showing the presence of catalase enzymes (Reiner, 2010).

Oxidase test. Filter paper was saturated in oxidase reagent (consists of 0.1 g oxidase reagent in 10 ml distilled water) and dabbed with bacterial culture. Change in color to purple within 10-60 seconds was considered positive (Shields & Cathcart, 2010).

Methyl Red and Voges Proskauer tests. Both tests were performed in MR-VP broth medium (peptone 7.0 g/L, dextrose 5.0 g/L, potassium phosphate 5.0 g/L). Inoculation of tubes containing 5 ml of autoclaved medium with

each of the strains was done and kept for 48 hours at 37° C. In the methyl red test, five drops of methyl red reagent were added to 3 ml of culture. The presence of red color was positive for mixed-acid fermentation while the absence of red color indicated negative mixed-acid fermentation (Sharma, 2013). In the Voges-Proskauer test, 3 ml of culture was mixed with 0.6 ml alpha-naphthol and 0.2 ml of 40% KOH for 15 minutes and observed for red color on the surface.

2.4 Physiological Characterization of Selenite Tolerance

Effect of temperature: Nutrient broth supplemented with 300 µl Na₂SeO₃ was inoculated with each strain and incubated separately at 28°C, 37°C, and 50°C for 48 h. Cultures were centrifuged (5000 rpm, 10 min), and the resulting pellet was washed with 10ml of sterile distilled water and resuspended in 5ml distilled water. Absorbance was recorded at 500nm against an uninoculated nutrient broth blank. (Abbas, 2012)

Effect of pH: Nutrient broth was adjusted to pH 5, 7, and 9 prior to autoclaving, then supplemented with 300µl Na₂SeO₃. Each strain was inoculated and incubated at 37°C for 48 h, followed by centrifugation, washing and optical density measurement as described above.

Tolerance to High Selenite Concentrations: Strains were cultured in nutrient broth containing sodium selenite (1000-2000 µg/ml) and incubated at 37°C for 48 h. Selenite reduction to elemental selenium was assessed both visually by red coloration of the culture and quantitatively by OD measurement.

2.5 Heavy Metal Resistance Profiling

Cross-resistance to heavy metals was determined by streaking the isolates on nutrient agar containing 30 µg/ml of each metal salt including Mercury (Hg), Cobalt (Co), Nickel (Ni), Copper (Cu), Manganese (Mn), and Zinc (Zn) and then plates were incubated at 37°C for 24 h.

2.6 Plant Growth-Promoting Characteristics

IAA production was measured by Salkowski spectrophotometric assay. The isolates were grown on nutrient broth media amended with

200 µg/ml L-tryptophan at 37°C in darkness for 48 h. Centrifugation was done at 10,000 rpm for 5 min, and 2 ml of supernatant was mixed with 1 ml Salkowski reagent consisting of 35% HClO₄ and 0.05 M FeCl₃. Reaction mixtures were kept in darkness for 30 min; the appearance of a pink to red color indicated the presence of IAA production that was measured at 535 nm (Lin *et al.*, 2022).

Isolates were streaked onto Pikovskaya agar plates amended with tricalcium phosphate as the only source of phosphorus and grown for 7 days at 37°C. Presence of a clear zone around colony indicates the ability to solubilize phosphate.

2.7 Biosynthesis of Selenium Nanoparticles

Selenium nanoparticles (Se-NPs) were produced biologically by inoculating each isolate into nutrient broth media sterilized with 500 µg/ml Na₂SeO₃ and incubated at 37°C for 48 hours (Pyrzynska & Sentkowska, 2022). The reaction between selenite and formation of elemental selenium was observed by changing the color of the nutrient broth to red. After 48 hours, samples were collected from the broth and centrifuged at 5000 rpm for 5 minutes. The resulting pellets were washed using autoclaved distilled water several times. For leaf sprays, 0.5 ml of each washed pellet was suspended in 50 ml of autoclaved distilled water.

2.8. Greenhouse Experiment

Lactuca sativa stem cuttings were surface-cleaned and grown at four plants per pot across 28 pots. Three experimental treatments were applied: bacterial inoculation, elemental selenium supplementation, and Se-NP foliar spray. Each with five replicate pots per bacterial strain, alongside one uninoculated control per treatment set. For bacterial inoculation, 3-4 loopfuls of each purified strain were suspended in 50ml sterile distilled water and agitated on a shaker (37°C, 2h) prior to application. For elemental selenium treatment, a 1% stock solution (0.5g selenium in 50ml sterile distilled water) was diluted (750µl stock into 15ml sterile distilled water) to prepare the working solution. Se-NP suspensions were applied as a foliar spray, ensuring uniform coverage of all leaflets. Bacterial inoculation and elemental selenium

treatments were repeated at 15-day intervals, while Se-NP foliar sprays were applied every 10 days, over the 6-7-week experimental period.

2.9. Biochemical Evaluation of Plant Growth Parameters

Total soluble protein in leaf tissue was determined at week seven following the method of Lowry. Frozen leaf samples were homogenized in phosphate buffer (pH 7), and the resulting supernatant was collected by centrifugation and chilled for 1h. Folin reagent (2ml) was added to 1.5ml of supernatant and incubated at room temperature for 15 min, followed by addition of Folin-Ciocalteu phenol reagent and a further 45 min incubation. After the addition of reagents, optical density was recorded by spectrophotometer at 750nm.

2.9.1. Chlorophyll and Carotenoid assay

Chlorophyll a, Chlorophyll b, and carotenoid content were determined spectrophotometrically from fresh leaf tissue extracted in acetone. Following centrifugation (13,000 rpm, 10 min), supernatant absorbance was measured at 662nm (chlorophyll a), 645nm (chlorophyll b), and 470nm (carotenoids), with pigment concentrations expressed as $\mu\text{g/g}$ fresh weight.

3. Results

3.1 Isolation, Screening and Identification of Selenium-Resistant Bacteria

Fifteen visually different bacterial strains which emerged from soil samples were tested using selenium-selective experimental conditions. The five isolates A1-SER to A5-SER showed immediate and strong red color development which occurred within 24 hours after they were grown in sodium selenite supplemented media. The visible change shows that selenite (Se^{4+}) gets reduced to elemental selenium (Se^0) through this process which enables these isolates to transform selenium into harmless nanoparticles. The isolates showed different red color intensity levels because A3-SER, A4-SER and A5-SER showed the strongest ability to reduce selenium. It indicated that they possessed better metabolic capacity to handle selenium toxicity. It was observed that bacterial colonies were developed through two pathways which produced circular

and irregular colony shapes with smooth and slightly rough surface patterns respectively. The scientific examination found that the selected isolates built their Gram-positive rod-shaped endospore structure which protected them from environmental conditions.

3.1.1 Biochemical Testing

The biochemical tests showed that all isolates possessed catalase activity which caused them to release gas bubbles instantaneously when they encountered hydrogen peroxide. The isolates displayed different oxidase activity levels, and the MR-VP tests demonstrated different metabolic activities as well, which confirmed that the selected strains had distinct physiological characteristics.

3.2 Physiological Response Under Environmental and Selenium Stress

3.2.1 Effect of Temperature on Bacterial Growth

All isolate growth patterns were assessed during tests which involved three temperature settings of 28°C, 37°C and 50°C while selenium stress was applied. It was found that bacterial growth was maximum at 28°C based on their optical density measurements which showed OD values between $0.82 \text{ nm} \pm 0.03$ and $0.91 \text{ nm} \pm 0.02$ for various strains. The growth rate at 37°C experienced a minor decrease which resulted in an OD_{500} value range of approximately 0.65 nm to 0.74 nm. The growth rate at 50°C showed a major decrease because the OD_{500} value fell below 0.40 nm. These effects are shown in the graphical representation in Figure 2. It was proved through statistical analysis that 28°C temperatures produced higher growth results than all tested temperature conditions. Moderate temperature provided maximum selenium reduction and bacterial metabolism because these conditions improve the enzymatic functions controlling redox processes.

3.2.2 Influence of pH on Growth and Selenium Reduction

It was observed that the bacteria grew the best at a neutral pH of 7. After proper observation it was found through the OD_{500} results, which were between 0.88 nm and $0.95 \text{ nm} \pm 0.03$. When the pH was too low at pH 5 (acidic) or too

high at pH 9(alkaline) the bacterial growth was not ideal and the OD₅₀₀ results were between 0.45nm and 0.62 nm as depicted in Figure 3.

This was important because the test results showed a difference between these two

conditions at optimal growth temperature. This means that the Selenium Reduction by these bacteria depends on the pH and that these bacteria can grow better in soil that has a neutral pH.

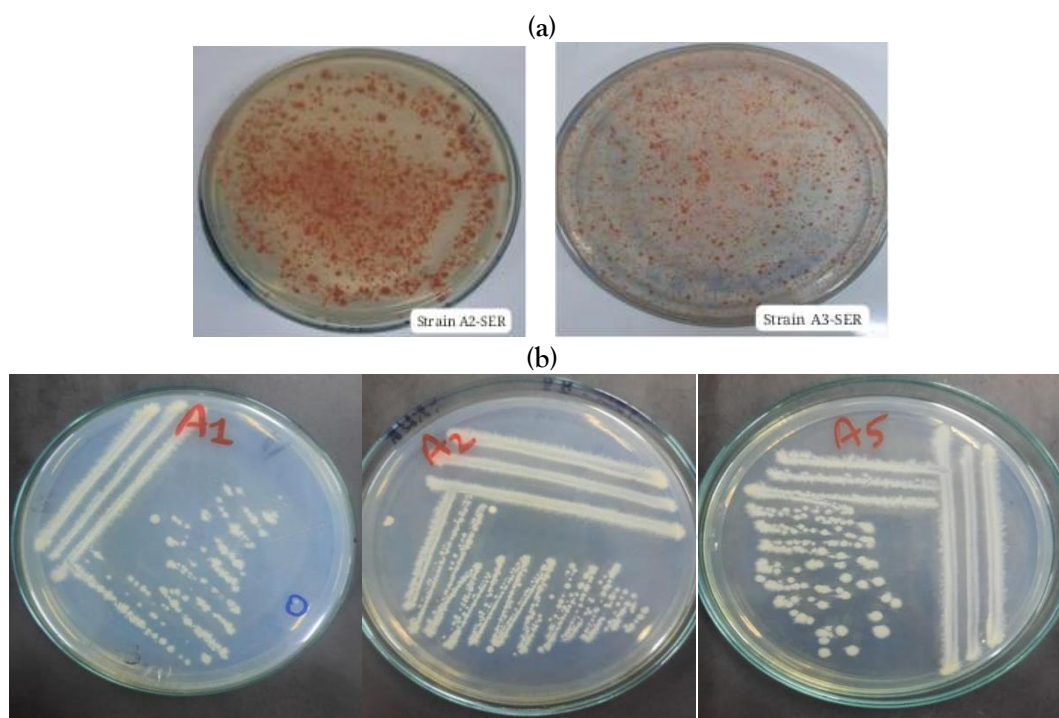


Figure: 1

(a) The figure above shows bacterial strains isolated after spreading on Selenite containing media. The strains showed red colored colonies which depicted Selenite reduction to Selenium.

(b) The figures show purified bacterial strains that were obtained after pure culturing and growing at an optimal temperature of 37°C for an incubation time of 24 hours.

3.2.3 Tolerance to Increasing Sodium Selenite Concentrations

The isolates showed exceptional tolerance toward all tested Sodium Selenite concentrations which ranged from 1000 to 2000 micrograms per milliliter. Growth was observed high up to 1600 micrograms per milliliter because at that level the OD₅₀₀ read over 0.70 nm ± 0.04. That result suggested strong detox work and good reduction ability as graphically shown in Figure 4.

After 1800 micrograms per milliliter, growth dropped and the OD₅₀₀ value fell to a range from 0.40 nm to 0.50 nm. This drop happened because selenium stress affected the organism and it failed to grow properly. At 2000 micrograms per milliliter, the sample showed a light red color with faint growth. Overall,

selenium is helpful at moderate amounts as a micronutrient but at higher levels it acts as an inhibitor. That is why it is described as a dose based or dual function element.

3.2.4 Heavy Metal Resistance Profile

The isolates showed different levels of resistance against six heavy metals including Mercury (Hg), Cobalt (Co), Nickel (Ni), Copper (Cu), Manganese (Mn), and Zinc (Zn). The presence of Nickel, Zinc and Manganese enabled the bacteria to grow at high rates which showed their strong resistance to these metals. The bacteria showed moderate growth pattern when exposed to Copper but Mercury and Cobalt caused complete growth inhibition as shown in Figure 5.

The qualitative assessment of colony growth

showed that resistance to Ni, Zn and Mn existed as a common feature among all isolates which proved the existence of shared detoxification pathways. The reduced tolerance to Hg and Co may be attributed to their higher toxicity and interference with essential cellular processes.

3.3 Plant Growth Promoting Characteristics

3.3.1 Indole Acetic Acid IAA Production

It was assessed that all isolates can produce Indole Acetic Acid (IAA), but their production levels increase when L-tryptophan is present. The analysis determined that IAA production reached a range between 21.3 ± 1.2 µg/ml and 45.50 ± 1.5 µg/ml. The A4-SER isolate produced the most IAA with a total of 45.50 µg/ml while A3-SER and A5-SER followed behind with lower concentrations. The statistical analysis demonstrated that A4-SER produced IAA levels which exceeded other strains at a p-value of less than 0.05. The elevated auxin levels which plants produce will develop roots while plants absorb nutrients from their environment.

3.3.2 Phosphate Solubilization

All isolates formed visible halo zones on Pikovskaya agar which proved their capacity to dissolve inorganic phosphate. The halo zones had a diameter which ranged between 4 mm to 9 mm while A3-SER and A5-SER produced larger zones of 9 mm which demonstrated their ability to solubilize substances more efficiently.

3.4 Biosynthesis and Application of Selenium Nanoparticles

The synthesis of selenium nanoparticles through biological methods was established when Sodium Selenite incubation caused the culture broth to change from its original colorless state to a brick red hue. The change of color shows that elemental selenium has developed into nanoparticle-sized particles.

The biogenic Se-NPs synthesis was achieved through visual verification together with successful pellet recovery from centrifugation despite forgoing advanced physicochemical analysis methods. The nanoparticle suspensions as foliar spray treatment was applied at 10-day intervals to achieve continuous plant tissue exposure and ongoing particle absorption.

3.5 Comparative Evaluation of Plant Growth Under Different Treatments

3.5.1. Fresh weight calculation

The growth patterns of plants were observed, and it was found that there were differences between the plant groups after six to seven weeks of germination. The plants that were treated with the Se-NPs foliar spray grew the best up to 18.6 grams. This was more than both plant groups that were supplemented with selenium and the plants with bacterial inoculation weighing 14.2 grams and 12.8 grams respectively. The SeNP foliar spray treatment helped increase the biomass accumulation of the plants. The fresh weight of plants is shown in Figure 6. Control plants showed the lowest biomass which measured 9.5 ± 0.4 g. Enhanced growth occurred in foliar-treated plants because their leaf surfaces absorb nutrients directly and the increased application frequency raised selenium content.

3.5.2 Chlorophyll Pigment Content

Chlorophyll analysis revealed a marked increase in pigment content in Selenium as well as foliar treated plants. The SeNP-treated plants had the highest chlorophyll A content which measured 4.901 ± 0.15 µg/g of fresh weight while selenium-treated plants had 3.85 ± 0.12 µg/g and bacterial inoculated plants showed 3.42 ± 0.10 µg/g. Chlorophyll B and carotenoids followed a similar trend, though at comparatively lower concentrations. SeNP-treated plants demonstrated better photosynthetic efficiency and improved physiological performance according to these results. The detailed comparison between pigment content can be observed in Figures 7, Figure 8 and Figure 9 through graphical illustrations.

3.5.3 Soluble Protein Content

Protein estimation revealed a significant increase in treated plants compared to control. The SeNP-treated plants had the highest protein content which resulted in absorbance values that measured approximately 1.8 times higher than control. Plants treated with A5-SER-derived nanoparticles reached the highest protein levels among strains which indicates that different strains produce varying effects with

their respective nanoparticles.

3.5.4 Morphological and Physiological Observations

Visual assessment revealed that SeNP-treated plants exhibited more vigorous and dense growth with improved leaf expansion, delayed

senescence and reduced wilting. The control plants displayed early stress symptoms through their decreased leaf size and their untimely drying process. The repeated foliar application of SeNPs at shorter intervals likely contributed to sustained nutrient availability and enhanced stress tolerance.

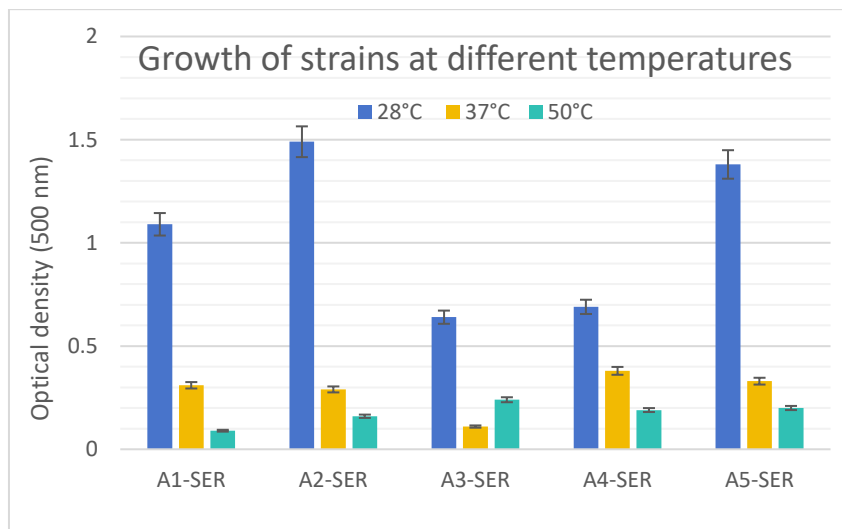


Figure 2: This figure shows the growth of strains at different temperatures. It can be inferred that the maximum growth of all strains is occurring at 28°C. The strains show varied growth at other higher temperature ranges of 37°C and 50°C.

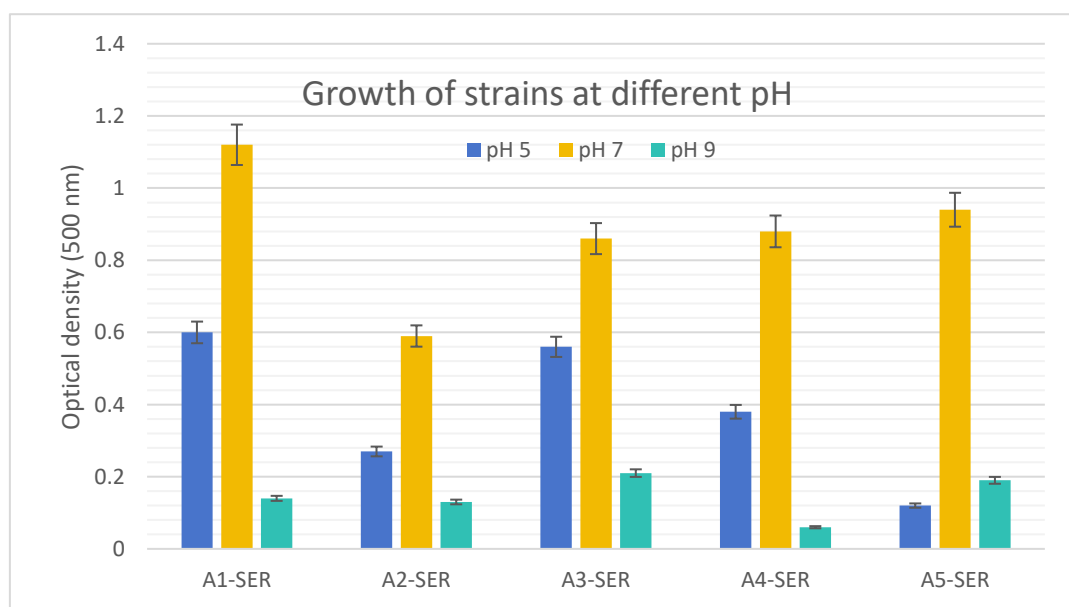


Figure 3: This graph shows how the strains grow at different pH values. It shows that the maximum growth is at pH 7 which is neutral pH while lower growth is observed at acidic and alkaline pH values of 5 and 9 respectively.

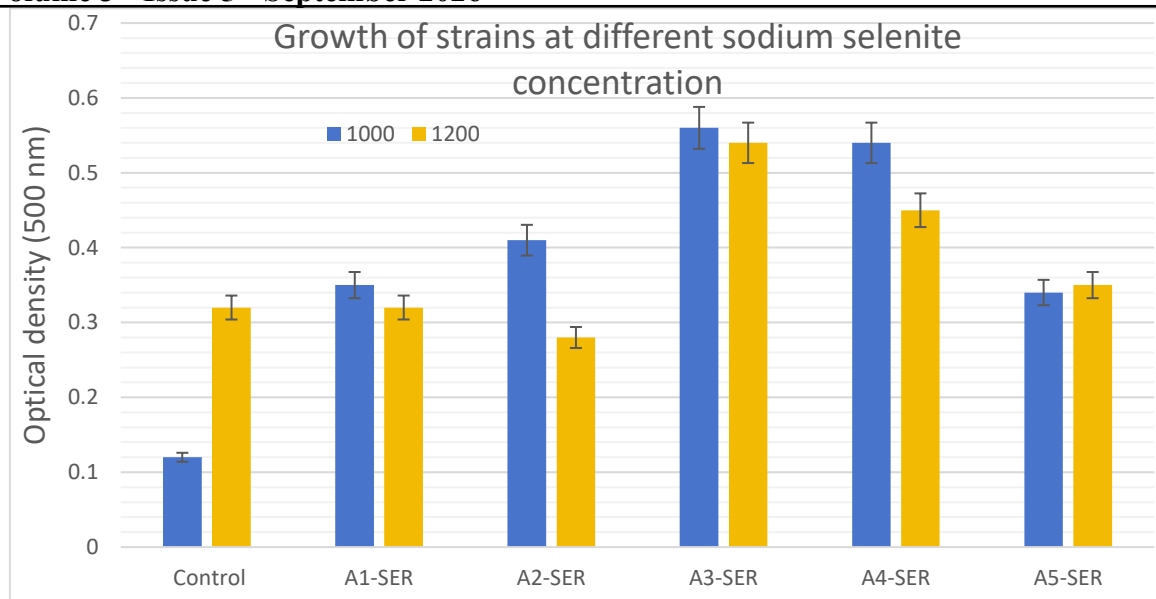


Figure 4: This figure shows how the selected strains grow at 1000 and 1200 µg/ml concentration of sodium selenite. Maximum reduction at the specified concentration by all the strains can be observed from the graph.

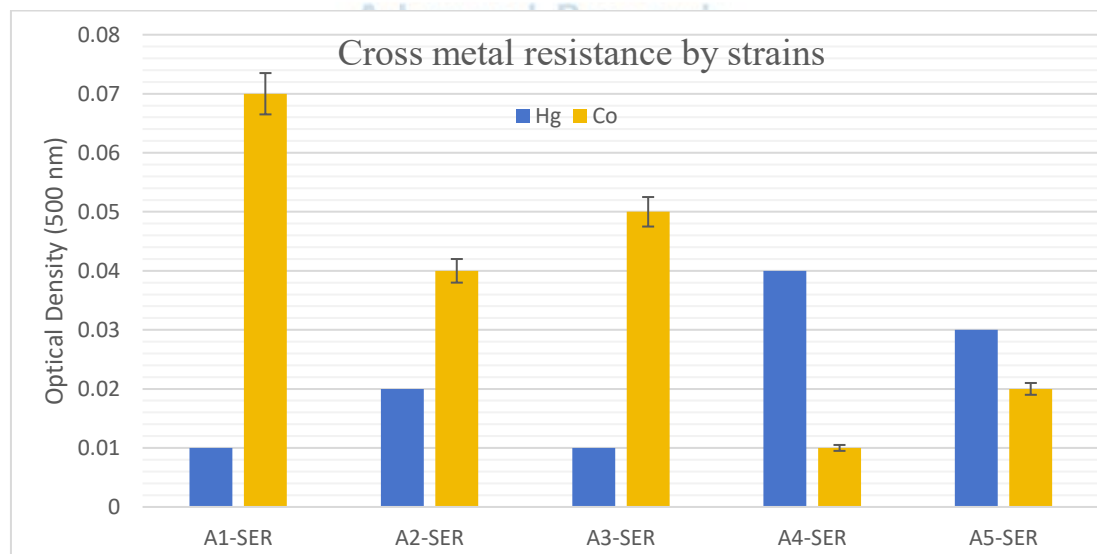


Figure 5: This figure shows the cross-metal resistance by all strains in the presence of metals such as Mercury (Hg) and Cobalt (Co). Varied resistance is shown by the strains in the presence of metals.

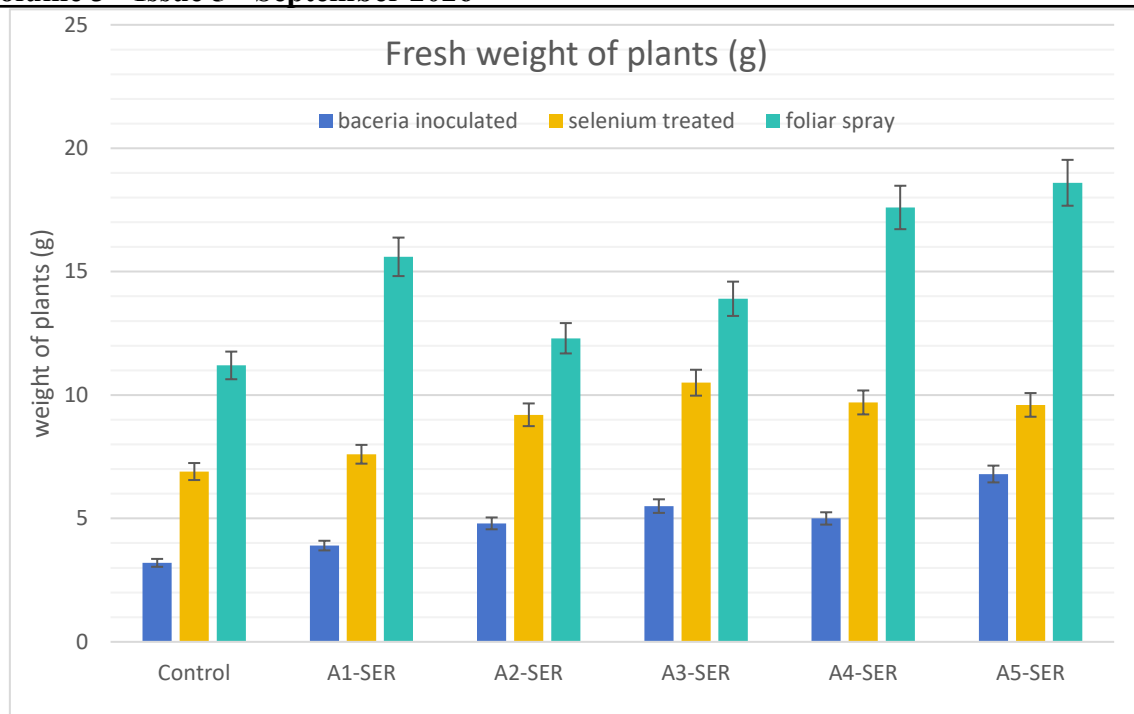


Figure 6: This figure shows the fresh weights of plants in grams after various treatments conducted on them such as bacterial inoculated plants, selenium treated plants and foliar sprayed plants. The foliar spray has the maximum effect showing increased weight of plants in comparison to other treatments.

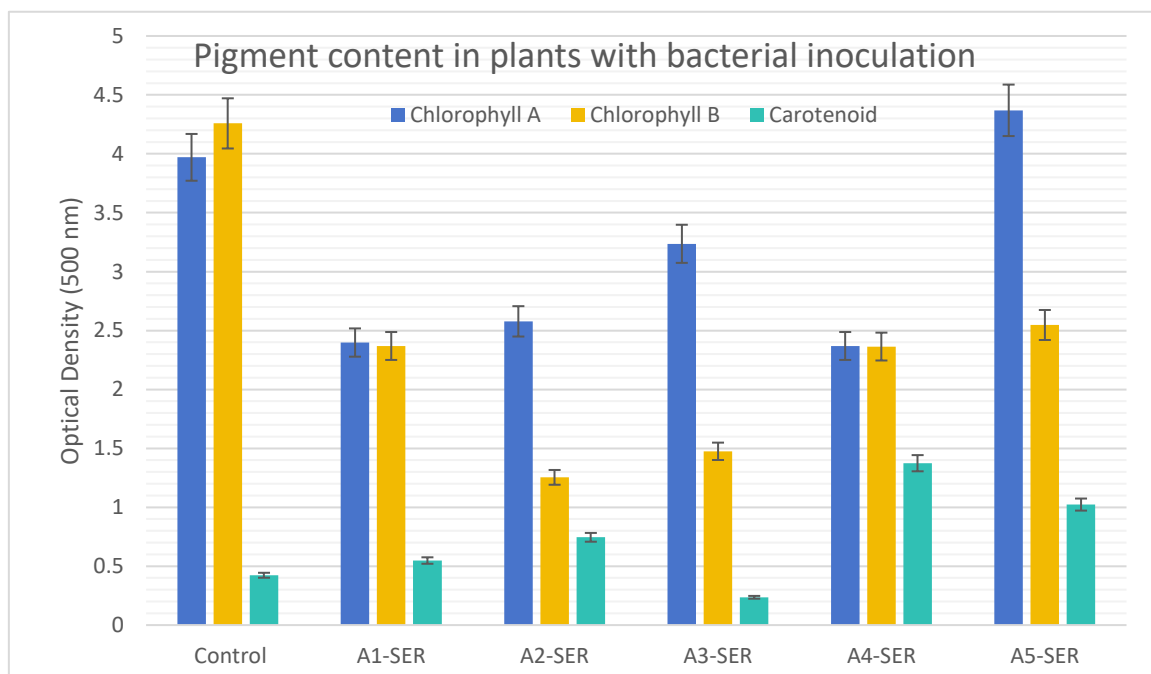


Figure 7: This figure shows the pigment content in plants after bacterial inoculation treatment. It is implicated that Chlorophyll a and Chlorophyll b have the maximum content while Carotenoids are lesser in comparison to both.

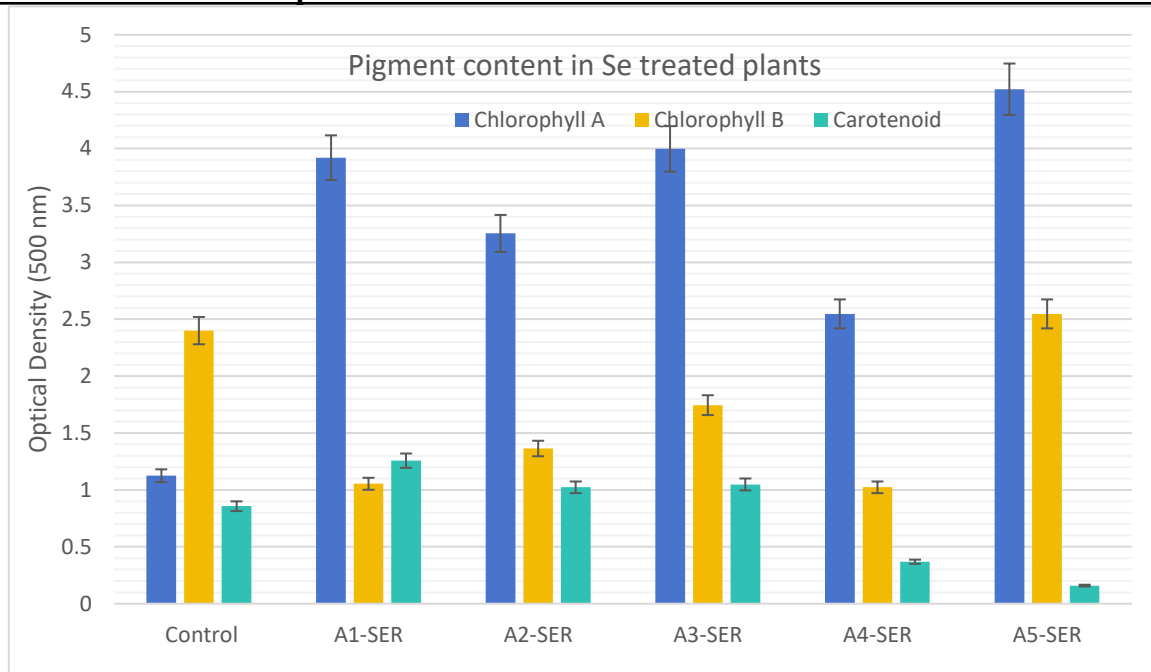


Figure 8: This figure shows the pigment content in plants after treatment with Selenium. It is implicated that Chlorophyll a has the maximum output content while Chlorophyll b and Carotenoids are present in lower quantity.

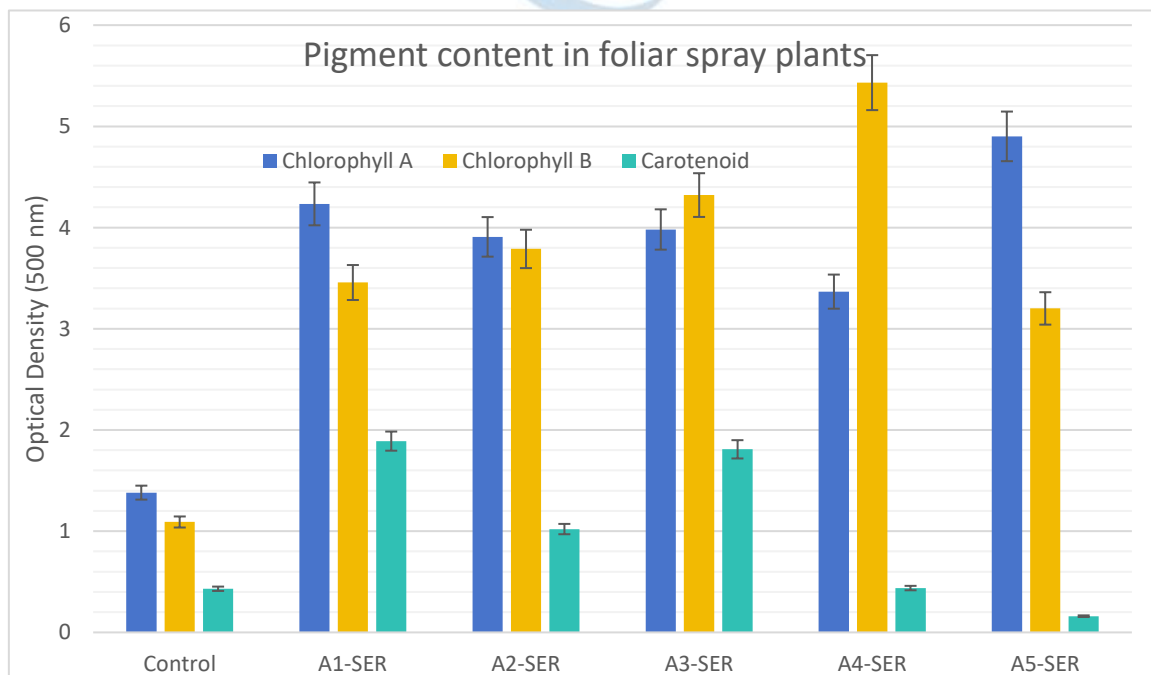


Figure 9: This figure shows the pigment content in plants after treatment with foliar spray. It is implicated that both Chlorophyll a and Chlorophyll b have the maximum concentration while Carotenoids are present in lower quantity.

4. DISCUSSION

The current research study assessed selenium-resistant bacteria based on their capacity to produce bio selenium nanoparticles (Se-NPs),

which function as plant growth promoters and increased selenium biofortification. The research results demonstrated that SeNPs produced through microbial processes provided

better biological value as compared to traditional fertilizers. It helped plants grow faster, better and withstand environmental stress.

The formation of red elemental selenium by the isolates confirmed their ability to reduce toxic selenite (Se^{4+}) into insoluble elemental selenium (Se^0). Soil bacteria detoxify toxins through a process which *Bacillus* species used to reduce oxidative damage by their reductive pathways. (Kushwaha *et al.*, 2022).

Selenite reduction process relies on cellular redox systems which involve thiol reactions and reductase enzymes. The bacterial isolates continued to grow at higher selenium levels which reached upto 1600 $\mu\text{g/ml}$ because their internal detoxification mechanisms protected against selenium. Previous research demonstrated that selenium-resistant bacteria can sustain their redox balance during exposure to metal stress environments. (Jiang, Wang, Zhao, & Peng, 2024)

The process of converting selenite into elemental selenium results in two outcomes which include the elimination of toxic effects and the creation of selenium nanoparticles which possess distinct physiochemical characteristics through their greater surface area and improved biochemical activity. Bacterial growth depends on environmental factors because selenium reduction processes showed similar environmental sensitivity. (Kushwaha *et al.*, 2022). The study showed that organisms grew at an optimum at 28°C and a neutral pH. Under those settings, enzymes helped in reducing selenite into selenium. When the physiological factors of environment shifted from the optimum, growth dropped and metabolic activity slowed down resulting in lesser selenite reduction and reduced growth. Sometimes changes in bacterial cell membrane also play a critical role affecting how materials are transported through the membrane. These findings were also similar to some studies where microbial selenium reduction slows when pH and temperature were increased. (Logan, Zhu, Lens, & Cetecioglu, 2023)

These isolates also resisted and showed growth in the presence of several toxic heavy metals, which suggested they might employ different metal detoxification pathways. The key defense

mechanisms include active efflux pumping, extracellular and intracellular sequestration, and enzymatic detoxification. (Feng, Wei, & Tu, 2013). In the cross-metal resistance tests, strains showed resistance against Ni, Zn, and Mn while in the presences of Hg and Co bacterial isolates showed no growth. This pattern fits with a selective response to metal stress in their surroundings. Researchers showed that microbial pathways which detoxify heavy metals follow the same pathways which enable selenium reduction (Logan *et al.*, 2023). The bacteria demonstrate dual functionality which helps them survive in their environment while making them effective agents for bioremediation and biofortification.

The isolates demonstrated the ability to function as plant growth-promoting rhizobacteria through their capability to produce Indole Acetic Acid and their ability to solubilize phosphate. The IAA production of A4-SER strain reaches high levels which enables the strain to create root systems that improve plant nutrient and water absorption ability. It depicts that plant growth effects extend beyond traditional PGPR growth mechanisms (Patani *et al.*, 2024). Bacterial inoculation also improved plant growth although the growth was less than when plants were applied selenium nanoparticles. It was evident that microbial activity needs to reach a specific level for optimal growth enhancement while selenium delivery method proves to be essential for growth results. The results of current study showed that SeNP foliar applications increased plant growth as compared to elemental selenium and bacterial inoculation. The nano-scale size of SeNPs allowed them to migrate efficiently through leaf cuticles and enter plant tissues directly. Foliar-applied nanoparticles have less soil-mediated constraints because soil selenium becomes subject to adsorption, leaching and microbial immobilization. The enhanced surface reactivity of nanoparticles enables their bioavailability to increase because nanoparticles can interact with cellular components more effectively. (Sanità, Carrese, & Lamberti, 2020). The presence of selenium led to increased chlorophyll content possibly chloroplast membrane stability and it also improved photosynthetic efficiency according to research findings. SeNPs serve a

critical function by controlling the antioxidant systems which exist in plants. Antioxidant enzymes which protect cells against oxidative damage include selenite because it functions as a constituent of glutathione peroxidase. The SeNP-treated plants show two beneficial effects which create an increase in protein content and a delay in plant aging process. The study results demonstrate essential findings which will help develop sustainable biofortification methods for future research. The usual ways of selenium supplementation often led to low efficiency and potential toxicity in plants. (Feng *et al.*, 2013). Biogenic Se-NPs provide a sustainable solution which delivers controlled and efficient results through environmentally friendly methods. This shows how integrating microbial processes with nanotechnology helps solve two key problems which affect agricultural productivity as well as the nutritional value of edible plants.

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5. CONCLUSION

This research study demonstrates a combination of microbiology, nanotechnology, and plant science to solve an ongoing agricultural and human dietary problem which involves selenium deficiency. The research isolates and identifies selenium-resistant bacterial strains which convert toxic selenite into elemental selenium to create a biological method for sustainable selenium nanoparticle (SeNP) production. The research results showed that foliar application of biogenic SeNPs produced better results than other methods. The study points to be used outside the lab having practical applications in wide ranges in life sciences. Biogenic nanoparticles could help raise crop yields and ease micronutrient shortages across many regions producing another possible benefit for public health. These advances could address selenium deficiency and in turn, people may eat better leading to improved health outcomes.

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