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SYNERGISTIC EFFECTS OF BIOGENIC SELENIUM NANOPARTICLES AND RHIZOBACTERIA ON GROWTH, PHYSIOLOGICAL, AND BIOCHEMICAL ATTRIBUTES OF LETTUCE

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Abstract

Sustainable agriculture requires innovative approaches to boost crop productivity and stress resilience while reducing environmental impact. Both plant growth-promoting bacteria (PGPB) and biogenic selenium nanoparticles (SeNPs) have shown considerable potential in enhancing plant performance, yet their combined effects on crop physiology and biochemistry remain underexplored.In this study, twenty selenium (Se)-resistant Gram-positive bacterial strains were isolated from agricultural soils and screened for key PGP traits, including nitrogen fixation, phosphate solubilization, and phytohormone production. Selected strains were used to inoculate Lactuca sativa (lettuce) seeds under semi-field conditions. Treatments included bacterial inoculation alone, foliar SeNP application alone, and their combined application.After six weeks, plant growth parameters (root length, leaf length, and fresh weight), pigment content (chlorophyll a, chlorophyll b, and carotenoids), soluble protein levels, and peroxidase activity were evaluated. The combined application of selected bacterial strains and SeNPs significantly enhanced lettuce growth and stress-related biochemical responses. Specifically, the NB11 + SeNP treatment increased soluble protein content by 75% and peroxidase activity by 65% compared to the control ($p \leq 0.05$). Heat map analysis revealed strong positive correlations between growth and biochemical traits under combined treatments, while principal component analysis (PCA) showed that the first three components explained 77.1% of the total variance, effectively distinguishing the combined treatments from other groups.These results indicate that the synergistic use of SeNPs and Se-resistant PGPB provides a promising bio-based approach to improve plant growth, stress tolerance, and nutritional quality. This strategy offers a sustainable alternative to conventional chemical fertilizers, particularly in stress-prone agroecosystems.

INTRODUCTION

The escalation of global food demand has imposed significant environmental and ecological challenges, requiring the development of innovative, sustainable methods to enhance crop productivity (Albahri et al., 2023). Traditional farming techniques based on excessive use of chemical fertilizers and pesticides not only lead to soil degradation but also loss of biodiversity and environmental pollution (Hossain et al., 2022). With the world shifting towards sustainable agriculture, the need for environmentally friendly, biological methods for enhancing crop production and stress management is building traction (Ejedegba, 2024). Plant growth-promoting bacteria

(PGPB) represent a diverse class of rhizosphere-associated microorganisms establishing a direct and indirect advantageous impact on plant growth and development (Oleńska et al., 2020). These include biological nitrogen fixation, phytohormone production (e.g., auxins, gibberellins), phosphate solubilization, and the mitigation of environmental stresses. Moreover, PGPB contribute to improved root morphology and nutrient uptake, thereby optimizing plant health and productivity (Vocciante et al., 2022).

The importance of selenium (Se), a micronutrient that is necessary for many living things, in plant growth and stress tolerance is widely acknowledged (Gui et al., 2022). Se exists in various forms and its oxidation states determine the bioavailability. Its oxyanions, in higher concentrations, may be toxic to all life forms (Kushwaha et al., 2022). By boosting antioxidant defenses, encouraging enzymatic activity, and controlling redox homeostasis, Se shields plants from oxidative damage brought on by environmental stressors such as heavy metals, salt, and drought (Lanza & Dos Reis, 2021). However, the bioavailability of Se in soils is often limited, presenting a challenge for effective supplementation (Liao et al., 2024).

Biogenic selenium nanoparticles (SeNPs), synthesized using eco-friendly, biological methods, offer a solution by delivering Se in a highly bioavailable and controlled manner, minimizing toxicity risks while maximizing efficacy (Zambonino et al., 2023). Due to their controlled release and lower toxicity, SeNPs are found to be ideal for targeted delivery within plant systems (Ikram et al., 2021). Research has demonstrated SeNPs' ability to enhance chlorophyll content, antioxidant enzyme activity, and overall plant resistance (Shahbaz et al., 2023).

The combination of PGPB and biogenic SeNPs introduces a novel paradigm in sustainable crop management. PGPB can facilitate the uptake and utilization of SeNPs by modulating the rhizosphere environment, while SeNPs can amplify the physiological benefits conferred by PGPB (Rajput et al., 2023). This synergistic interaction holds the potential to enhance plant growth, photosynthetic efficiency, nutrient acquisition, and overall biomass accumulation (Verma et al., 2024). Furthermore, the integration of these two agents may activate stress-responsive pathways, conferring enhanced resilience against biotic and abiotic stress factors (Ranjan et al., 2024). While there are promising individual advantages of PGPB and SeNPs, little work has investigated their synergistic use. It is unknown how co- treatment with Se-resistant bacterial strains and foliar application of biogenic SeNPs can collectively impact plant's physiological and biochemical reactions under open-field conditions. The capacity of rhizobacteria to regulate Se uptake, mitigate its toxicity, and promote SeNP utilization has not been studied in detail.

This study aims to evaluate the synergistic effect of Se- reducing, gram-positive PGPB strains in conjunction with biogenic SeNPs to promote growth and stress resistance in *Lactuca sativa* (lettuce). To our knowledge, this is one of the first studies to assess these interactions comprehensively under semi-field conditions, with in-depth consideration of both physiological (root/shoot biomass, pigment production) and biochemical (antioxidant enzyme, protein levels) markers.

Through this investigation, the research contributes to the growing field of nano-enabled agriculture, highlighting the potential of integrating microbial biotechnology and nanotechnology to develop scalable, environmentally friendly solutions for modern crop production systems.

1. Materials and methods

Twenty gram-positive, Se-resistant strains were isolated from agricultural soil from the botanical garden of Punjab University, Lahore. Among them, ten isolates (NB02, NB03, NB04, NB05, NB06, NB10, NB11, NB14, NB17, and NB19)

were chosen for further study considering their Se-reducing activity and plant growth-promoting activity. Red colonies on nutrient agar containing 1% sodium selenite (Na_2SeO_3) identified the strains to be reducing selenite (Tavafi et al., 2023). To determine the strains' physical and biochemical characteristics, pure cultures were subjected to biochemical testing including phosphate solubilization, oxidase, and catalase and extent for nitrate reduction, ammonia synthesis, and auxin production were validated by important assays (Shahdin et al., 2024).

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1.1. Synthesis and characterization of biogenic SeNPs

With few modifications in (Sans et al., 2023) for the synthesis of nanoparticles, isolated bacterial strains were grown in nutrient broth with 1mM sodium selenite. The cultures were incubated at 37°C, 150 rpm for 48 h. Appearance of red color revealed SeNP formation. The biomass was centrifuged at 10,000 rpm for 15 min, washed three times with distilled water, and ultrasonicated (40 kHz, 10 min) to liberate SeNPs.

1.2. Plant growth conditions and experimental designs

Healthy lettuce plant seeds for the plant growth experiments were obtained from Sky Seeds Company, Lahore. The seeds variety was not indicated by the supplier, therefore it could not be mention in the study. The seeds were sterilized using a solution of mercuric chloride (0.1%), soaking for five minutes, and then washed with autoclaved distilled water. For seeds inoculation, bacterial suspensions were prepared at an optical density (600 nm) of 1.0 and lettuce seeds were soaked in the suspension for 20 to 25 min.

1.3. Experimental design

Pot experiment was performed in triplicate with a randomized complete block design (RCBD) under semi- controlled conditions (March–May 2024, ambient temperature: 18–42°C) at the Institute of Microbiology and Molecular Genetics, Wire House, University of the Punjab, Lahore. Sterilized fertile loamy soil filled in sixty-six pots (15 cm diameter). Three plant treatment groups were established using potted lettuce seeds (approximately 10-12 seeds per pot), each with respective controls:

1. Control (non-inoculated, water treatment only)
2. Soil inoculation with bacterial suspension
3. Bacterial inoculation combined with foliar application of biogenic SeNPs
4. Bacterial inoculation combined with selenite supplementation

Each group had three replicates for each bacterial strain. For foliar treatment, SeNPs were sprayed twice (days 10 and 20 after germination) at a rate of 10 mL per plant using a fine spray bottle (Figure 2).

Figure 1: Indicating the growth pattern of plants after five weeks under different treatments i.e., Bacterial inoculated plants (inoculation of bacterial suspension on soil)(right), Inoculated plants + foliar application of biogenic selenium nanoparticles(left) and Inoculated plants + selenite treatment (middle) as compared to control.

1.4. Growth parameters of plants

Number of plants/pot, plants fresh weight, measurements of leaves length, roots length and number of roots per plant were assessed. Fresh leaves from 6–7 weeks old plants were obtained, grinded and one gram of it was crushed with 5ml of 80% acetone. The resultant mixture was centrifuged for 10 minutes at 5000 rpm, the supernatant was collected (Lester et al., 2004). The optical density of the supernatant was recorded at 663 nm, 645 nm, and 470 nm for chlorophyll 'a', and 'b' and carotenoids, respectively. Additionally, they were

quantified using Arnon's (1949) equation (Porra, 2002).

Chl 'a' $(mg/g) = [(12.7 \times A^{663}) - (2.6 \times A^{645})]$

Chl 'b' (mg/g) = $[(22.9 \times A^{645}) - (4.68 \times A^{663})]$ *CX+c = 1000* A_{470} *- 1.90 Chl 'a' - 63.14 Chl 'b' / 214*

1.5. Enzyme extract for peroxidase and total soluble protein content.

Fresh leaves were frozen for 24 hours and crushed with polyvinylpyrrolidone (PVP) and phosphate buffer and then centrifuged to obtain a solution. The extract was kept at 0°C for the analysis of its protein content and peroxidase activity (Maehly & Chance, 1954).

1.6. Total soluble protein content

The total soluble protein concentration was estimated by (Racusen & Johnstone, 1961) with some modifications. About 2 ml of Biuret's reagent was mixed with 0.2 ml of enzyme extract and kept at room temperature for 30 minutes, and optical density was recorded at 445 nm using a microplate reader. The total protein content of a solution was indicated by blue-green coloration.

1.7. Peroxidase quantification in lettuce plant

After mixing 200 µl of enzyme extract with 2 ml of 0.1M phosphate buffer solution, the mixture was allowed to sit at room temperature for 25 minutes, and then 200 µl of 0.1% guaiacol solution was added. Following the incubation period, 100 µl of 0.3% H2O2 solution was added. Phosphate buffer and 200 µl of $H2O_2$ were combined to create the blank test solution. A microplate reader was used to measure the optical density at 470 nm as soon as the peroxide solution was added.

1.8. Statistical analysis

All the experimental data were analyzed using one-way analysis of variance (ANOVA) to establish the influence of bacterial inoculation, SeNPs foliar application, and their combinations on the parameters measured for growth and biochemistry. Duncan's Multiple Range Test (DMRT) was used with a significance level of $p \leq 0.05$ for mean comparison. Principal component analysis (PCA) was applied to determine multivariate patterns and the major sources of variation between the physiological and biochemical characteristics. A heat map was created to graphically display the relative differences in treatment effects for all the variables that were measured. Statistical analysis was carried out with IBM SPSS Statistics (Version 23), and graphical representations were prepared with Microsoft Excel.

2. Results

2.1. Physiological growth parameters

The effects of foliar SeNPs treatment and bacterial inoculation were assessed using growth indices and physiological indicators. However, in third group seeds treated with sodium selenite during field trials, the germination was hindered with the exception of seeds that sprouted in the pot marked NB19 but later stunted seedling growth was observed. Conversely, as compared to untreated controls, plants treated with bacterial strains showed better growth metrics, such as longer shoots and more plants per pot.

There were considerable differences ($p \leq 0.05$) among treatments for all the growth parameters that were measured. The combined application of selenium-resistant bacterial strains with biogenic SeNPs caused the maximum improvement in root length, leaf length, and fresh weight than those under single treatments and control. For example, among the treatments, NB14 (bacterial treated) recorded the highest number of plants per pot (12), an improvement of 67% from the control (4 plants). Similarly, NB05 + SeNP also resulted in 12 plants, comparable to NB14 based on Duncan's Multiple Range Test (DMRT). NB02, NB10 and NB11 displayed an increase in number of plants per pot in combined group (PGPB+SeNPs) with 9, 10 and 9 plants respectively as compared to 7, 6 and 4 plants per pot in group

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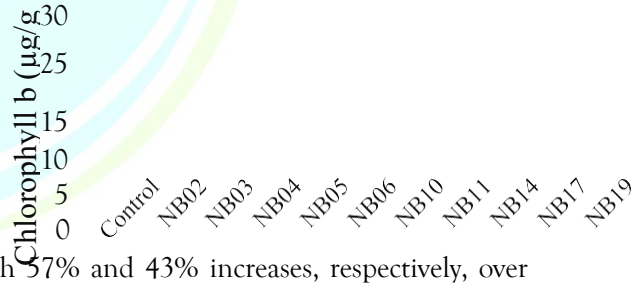
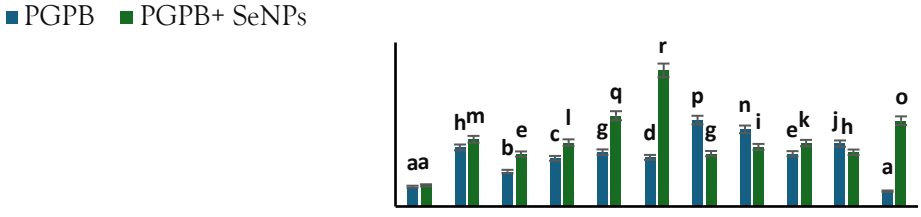
Table 1: Impact of Rhizobacterial Inoculation and Selenium Application on Growth Characteristics of Tomato Plants at Vegetative Stage: Values represent the mean (n = 3) ± standard deviation for the number of plants per pot, root length, leaf length, and fresh weight. In each column, numbers followed by lowercase letter[s] are significantly different judged from one- way ANOVA followed by Duncan multiple range test [DMRT], (P=0.05).

Bacterial strains	No of plants per pot		Root length		Leaf length		Fresh weight of plants	
	PGPB	PGPB+SeNPs	PGPB	PGPB+SeNPs	PGPB	PGPB+SeNPs	PGPB	PGPB+SeNPs
control	4±1 ^{a}	4±1 ^{a}	7±0.05 ^{a}	9±0.05^{b}	7±0.05^{a}	7±0.05^{b}	1.5±0.05^{a}	2.6±0.05^{d}
NB02	7 ±1^{bc}	9 ±1de	7±0.05^{b}	8±0.05^{c}	9±0.05^{d}	7±0.05^{b}	4.3±0.05^{l}	4.8±0.05^{m}
NB03	7 ±1^{bc}	4 ±1^{a}	10±0.05^{e}	10±0.05^{e}	8±0.05^{c}	7±0.1^{b}	2.1±0.05^{b}	3.5±0.05^{i}
NB04	8 ±1^{cd}	4 ±1^{a}	9±0.1^{d}	7±0.05^{b}	9±0.1^{d}	6±0.05^{a}	2.4±0.05^{d}	3.5±0.02^{i}
NB05	6 ±1^{b}	12 ±1^{f}	11±0.05^{f}	8±0.1^{c}	10±0.05^{e}	10±0.1^{e}	2.3±0.05^{c}	2.8±0.05^{f}
NB06	6 ±1^{b}	6 ±1^{b}	9±0.05^{d}	9±0.05^{d}	8±0.1^{c}	10±0.13^{e}	2.8±0.05^{f}	3.7±0.02^{j}
NB10	6 ±1^{b}	10 ±1^{e}	8±0.05^{c}	8±0.05^{c}	11±0.1^{f}	6±0.1^{a}	3.0±0.05^{g}	2.5±0.05^{e}
NB11	4 ±1^{a}	9 ±1de	6±0.05^{a}	9±0.05^{d}	7±0.05^{b}	9±0.05^{d}	2.1±0.02^{b}	3.2±0.05^{h}
NB14	12 ±1^{f}	7 ±1^{bc}	7±0.1^{b}	10±0.1^{e}	7±0.1^{b}	9±0.1^{d}	2.4±0.02^{d}	2.5±0.02^{e}
NB17	4 ±1^{a}	4 ±1^{a}	6±0.05^{a}	10±0.05^{e}	7±0.05^{b}	7±0.13^{b}	2.4±0.03^{d}	4.3±0.05^{l}
NB19	7 ±1^{bc}	7 ±1^{bc}	7±0.05^{b}	8±0.05^{c}	7±0.13^{b}	8±0.1^{c}	1.5±0.05^{a}	3.9±0.05^{k}
Treatment (p value)	0.000							

PGPB: Plant growth promoting rhizobacteria; **SeNPs:** Selenium Nanoparticles
PGPB+SeNPs: Combination treated with bacteria alone. Different letters in tables and figures refer to significant differences (Table 1).

With the exception of strains NB10 and NB19, plants in the foliar-treated groups had visible increased physiological characteristics overall. However, the treated plants maintained better growth and did not exhibit wilting as compared to untreated controls under normal greenhouse conditions. In comparison, the highest root lengths occurred in NB05 (bacteria alone, 11 cm) and NB03 + SeNP (10 cm), both 57% and 43% increases, respectively, over control (7 cm). Leaf length was greatest in NB05 + SeNP (10 cm) and NB06 + SeNP (10 cm), both 43% increase over control (7





Bacterial strains

cm). Within bacterial-only treatment groups, the most significant increase occurred in NB10 (11 cm). Fresh weight was maximized in the NB02 + SeNP group (4.8 g), being 69% greater compared to the control (2.6 g). The second most elevated weights

observed were in NB17 + SeNP (4.3 g) and NB06 + SeNP (3.7 g), with 65% and 42% increases, respectively. Within bacterial groups alone, NB10 (3.0 g) was 50% heavier than control. (Table 1).

2.2. Photosynthetic pigments content

All bacterial strains increased the content of photosynthetic pigments Chl "a" and Chl "b" (Figures 2 and 3). Bacterial strains NB10 and NB11 with SeNPs displayed 60% to 70% increase in pigment content with same trend for carotenoids. Although strains such as NB19 displayed the lowest increase amid the treated plants, they were nonetheless higher than the control. Some bacterial strains, particularly NB06, had discernible effects on the degree of enhanced pigment production when combined with biogenic Se-NPs as for chlorophyll a, the value comprises of 29.9 mg/g as compared to 16.3 mg/g in NB02 alone marking a 44% increase while in chlorophyll b, with value 24.9 mg/g as

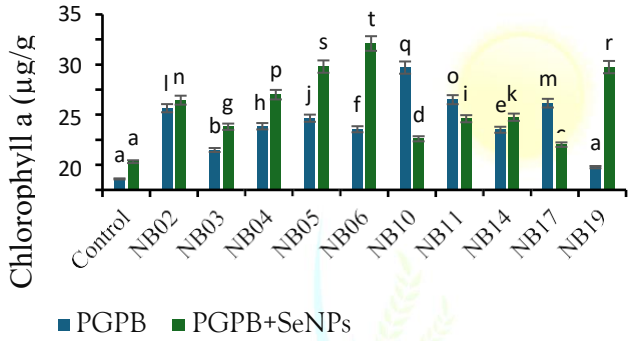
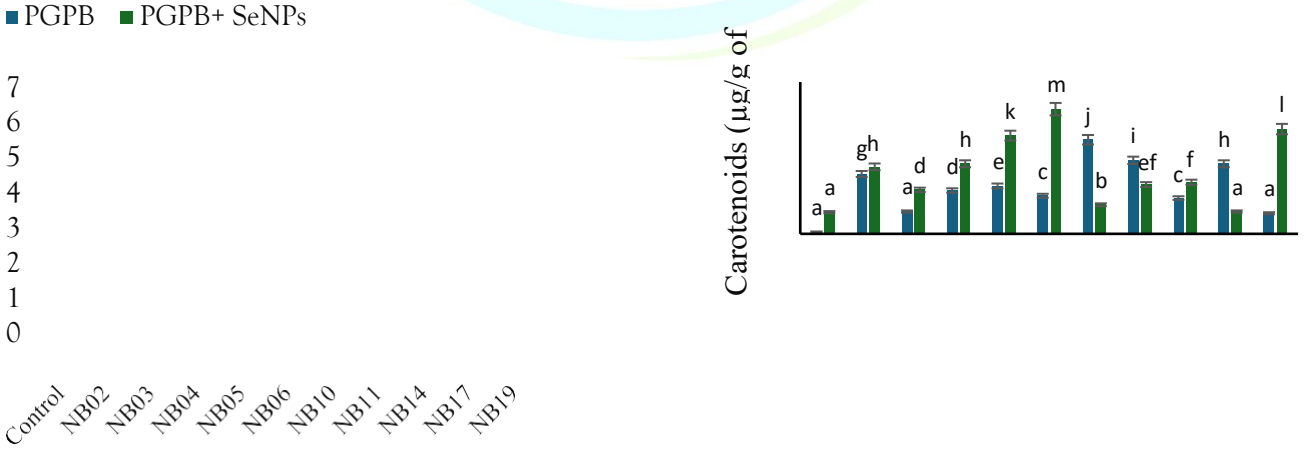


Figure 3. Chlorophyll b contents (mean ± SD) in bacterial and combined (PGPB + SeNPs) treated plants with different letters indicating significant differences (DMRT, $p \leq 0.05$).

compared to 10.8mg/g of control displayed 56% increase. The concentration of carotenoids showed 94% increase having 5.8mg/g as compared to 0.1 mg/g of control. ANOVA analysis revealed significant treatment effects on chlorophyll a, chlorophyll b, and carotenoid content ($p \leq 0.05$). DMRT categorized NB10 + SeNP and NB11 + SeNP as the best- performing treatments with significantly greater pigment content. (Figure 4).



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Bacterial strains

Figure 4. Carotenoids contents (mean ± SD) in bacterial and combined (PGPB + SeNPs) treated plants with different letters indicating significant differences (DMRT, $p \leq 0.05$).

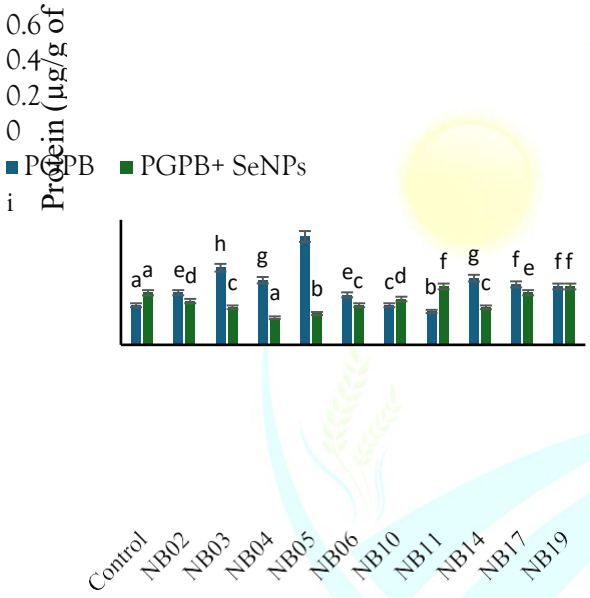
Bacterial strains

Figure 2. Chlorophyll a content (mean ± SD) in bacterial and combined (PGPB + SeNPs) treated plants with different letters indicating significant differences (DMRT, $p \leq 0.05$).

2.3. Biochemical growth parameters

When sprayed on leaves with SeNPs, the bacterial- treated seeds, particularly NB05 (0.52 mg/g), showed the greatest increase in soluble protein content of 71% as compared to control (0.25 mg/g) and other bacterial strains. Total soluble protein and peroxidase activity were significantly enhanced under the combined treatments.

NB11
+ SeNP treatment enhanced soluble protein content by 75%



Bacterial strains

2.4. Heat map visualization

A heat map was used to present the relative impacts of various treatments on all the measured factors. The heat map illustrated that the PGPB + SeNP combined treatment group grouped invariably in the higher value for growth, pigment content, and biochemical markers, indicating their better performance over individual treatments and the control (Table 2).

2.5. Principal component analysis (PCA)

Figure 5. Protein contents (mean ± SD) in bacterial and combined (PGPB + SeNPs) treated plants with different letters indicating significant differences (DMRT, $p \leq 0.05$).

compared to the control and peroxidase activity by 65% ($p \leq 0.05$) (Figure 3). For instance, NB05 + SeNP recorded 0.27 mg/g peroxidase content, 69% above the control (0.16 mg/g), and 42% greater than NB05 alone (0.12 mg/g) (Figure 6). These findings indicate that the combined application significantly enhanced growth and antioxidant defense systems of *Lactuca sativa*. Overall, foliar-supplied SeNPs increased the total soluble protein content more than bacterial inoculation alone, except NB11, NB17, and NB19 in the inoculated plant group, which showed a less significant difference (Figure 5).

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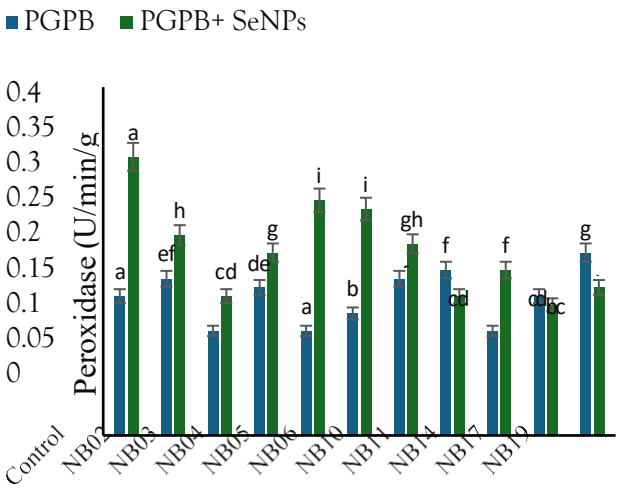


Bacterial strains

Figure 6. Peroxidase contents (mean ± SD) in bacterial and combined (PGPB + SeNPs) treated plants with different letters indicating significant differences (DMRT, $p \leq 0.05$).

Principal Component Analysis (PCA) was conducted to evaluate the multivariate pattern of the data. Kaiser-Meyer- Olkin (KMO) measure of sampling adequacy was 0.602, and Bartlett's test of sphericity was significant ($\chi^2 = 488.713$, $p < 0.001$), validating that the dataset was appropriate for PCA. Three principal components with eigenvalues > 1 accounted for a total cumulative proportion of 77.1% of the variance. PC1 explained 44.2% of the variance and was significantly correlated with chlorophyll a, chlorophyll b, and carotenoid content. PC2 (19.5%) correlated primarily with leaf length, root length, and protein content, while PC3 (13.4%) correlated with the number of plants per pot. The PCA biplot indicated distinct grouping of the combined treatments (e.g., NB10 + SeNP and NB11 + SeNP), reflecting a unique multivariate response pattern relative to single treatments and the control (Table 2).

3. Discussion

The escalating demand for sustainable agriculture has been driving interest in using microbial bioinoculants and nanotechnology, especially biogenic SeNPs, to promote plant growth and tolerance. Although studies have looked at the sole application of PGPB or SeNPs, few have explored the synergistic application of Se-reducing rhizobacteria and SeNPs in leafy greens such as *Lactuca sativa*. Moreover, previous studies have sometimes been deficient in thorough knowledge of biochemical responses, PCA-based evaluation, and comprehensive comparison of bacterial strains under SeNP foliar treatment. The present research fills these gaps by assessing the synergy between bacterial inoculation and SeNP foliar application in influencing lettuce's physiological, biochemical, and growth characteristics (Figure 7).

The increase in fresh weight, leaf and root length, and plant number per pot observed implies that SeNPs can possibly function as micronutrient enhancers and stress mitigators when applied together with PGPB strains like NB10 and NB11. This aligns with the recent studies that indicated that SeNPs, if applied in bioavailable form, decrease oxidative stress and improve plant nutrient absorption efficiency (Wang, et al., 2023b). The increased

Table 2: Pearson correlation coefficients between growth, biochemical, and pigment parameters of lettuce plants inoculated with selenium-resistant plant growth-promoting bacteria and biogenic selenium nanoparticles

	Protein content	Peroxidase content	No of plants per pot	Root length	Leaf length	Fresh weight	Chl a	Chl b	Carotenoid

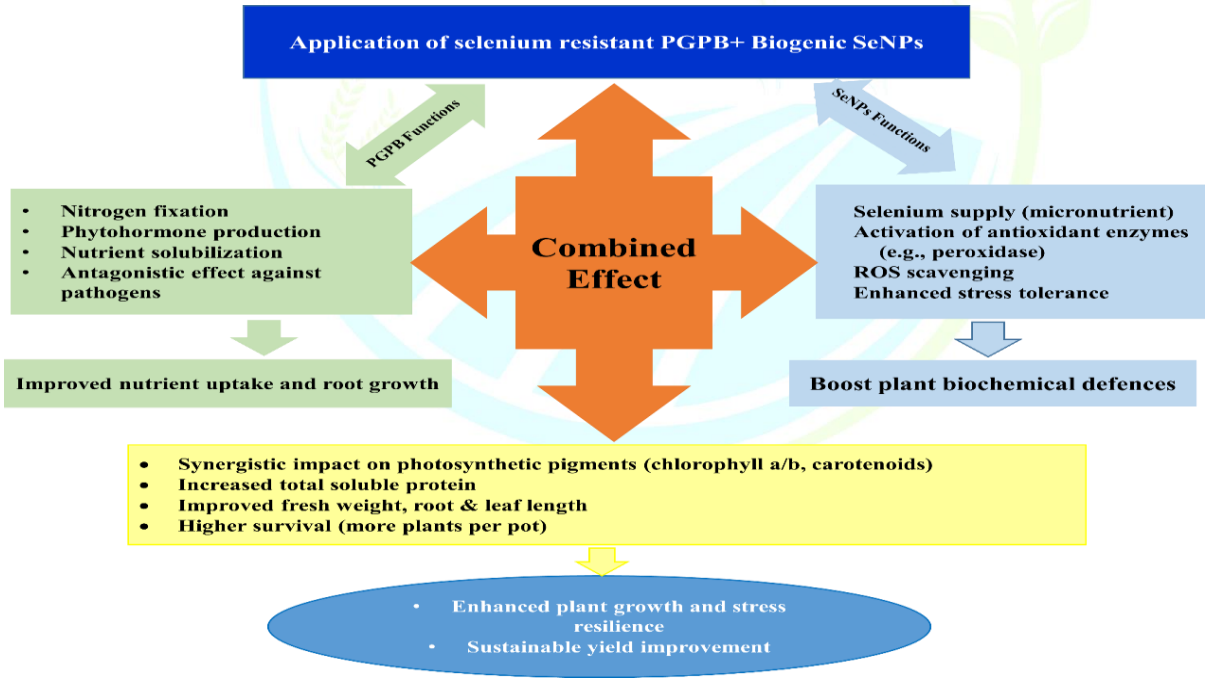
Protein contetn	Pearson Correlation	1	-.677**	092	375**	172	-.310*	-.391**	-.408**	-.399**
	Sig. (2-tailed)		000	486	003	188	016	.002	.001	.002
	N	60	60	60	60	60	60	60	60	60
Peroxidase content	Pearson Correlation	-.677**	1	204	-.301*	076	216	.508**	.525**	.558**
	Sig. (2-tailed)	.000		118	019	563	098	.000	.000	.000
	N	60	60	60	60	60	60	60	60	60
No of plants per pot	Pearson Correlation	092	204	1	-.053	164	-.103	-.005	-.015	-.034
	Sig. (2-tailed)	486	118		687	211	434	.972	.910	.795
	N	60	60	60	60	60	60	60	60	60
Root length	Pearson Correlation	375**	-.301*	-.053	1	366**	090	-.197	-.071	-.258*
	Sig. (2-tailed)	003	019	687		004	495	.131	.592	.047
	N	60	60	60	60	60	60	60	60	60
Leaf length	Pearson Correlation	172	076	164	366**	1	002	.497**	.461**	.469**
	Sig. (2-tailed)	188	563	211	004		989	.000	.000	.000
	N	60	60	60	60	60	60	60	60	60
Fresh weight	Pearson Correlation	-.310*	216	-.103	090	002	1	.395**	.405**	.341**
	Sig. (2-tailed)	016	098	434	495	989		.002	.001	.008
	N	60	60	60	60	60	60	60	60	60
Chl a	Pearson Correlation	-.391**	508**	-.005	-.197	497**	395**	1	.934**	.984**
	Sig. (2-tailed)	002	000	972	131	000	002		.000	.000
	N	60	60	60	60	60	60	60	60	60
Chl b	Pearson Correlation	-.408**	525**	-.015	-.071	461**	405**	.934**	1	.915**
	Sig. (2-tailed)	001	000	910	592	000	001	.000		.000
	N	60	60	60	60	60	60	60	60	60
Caroteno id	Pearson Correlation	-.399**	.558**	-.034	-.258*	469**	341**	984**	915**	1
	Sig. (2-tailed)	002	000	795	047	000	008	000	000	
	N	60	60	60	60	60	60	60	60	60

peroxidase activity and soluble protein content observed in SeNP-treated plants further indicate that Se is an important component of stress tolerance mechanisms (Ahmad et al.,2024). Our findings corroborate this, since co-application (NB11 + SeNP) elevated total soluble protein by a maximum of 75% and peroxidase activity by 65% relative to the control, as established by ANOVA and Duncan's Multiple Range Test (DMRT).

The differential response of bacterial strains indicates that some PGPB strains have different levels of interaction with SeNPs, probably because of their different metabolic pathways and capacity to modulate the uptake of Se (Verma et al., 2024). Additionally, SeNPs foliar application followed by bacterial inoculation had a greater biomass increase than the treatments applied individually. This

concurs with existing literature indicating that nanoparticles, in association with plant beneficial microbes, provide a microenvironment that enhances nutrient uptake and stress response pathways (Ranjan et al., 2024). Surprisingly, whereas the majority of bacterial strains promoted plant growth, there were specific combinations, like NB19, that had comparatively lower performance values, showing that not all PGPB strains have positive interactions with Se supplementation. This highlights the importance of targeted microbial strain selection when combining nanotechnology with sustainable agriculture practices (Karnwal et al., 2023).

The heatmap plot graphically illustrated uniform improvement across several physiological and biochemical characters, indicating the better performance of combined treatments compared to single treatments. This trend was quantitatively confirmed by the PCA, showing that the first three principal components explained 77.1% of the overall variance with distinct clustering of combined treatments. Parallel application of PCA to multivariate trait analysis provides further evidence for the utility of this method to depict variation induced by treatment (Hafez et al., 2019). Additionally, the Pearson correlation matrix showed strong positive correlations among major growth and pigment traits and thus suggested that photochemical capacity improvements were directly linked with increased biomass production and stress resistance. This observation reaffirms the value of integrated strategies for achieving sustainable crop yield enhancement. Furthermore, the Pearson correlation matrix showed high positive correlations between important growth and pigment characteristics, suggesting that increments in photosynthetic power were strongly linked with improved biomass generation and defense against stress. This observation supports the need for integrated strategies towards sustainable improvement of crop yields.



Also, the germination inhibition in sodium selenite-treated seeds indicates that though Se in nanoparticle form is useful, ionic selenium at increased doses can be harmful as reported earlier (Kushwaha et al., 2022). The interaction between SeNPs and PGPB is probably mediated by several mechanisms, such as increased nutrient solubilization, antioxidant enzyme activity, and root architecture (Mageshwaran et al.,2023). Earlier research emphasized that beneficial microorganisms could alter root exudates, which

further affects nanoparticle uptake and utilization efficiency. Moreover, the involvement of PGPB in modulating Se bioavailability through microbial metabolism also endorses their potential application as biofertilizers in precision agriculture. This synergistic interaction is especially vital in combating nutrient deficiencies in worn-out soils and hence is a viable option for sustainable agriculture (Asan, 2024). The capacity of SeNPs to induce stress-responsive pathways indicates that their use would be especially valuable in environments that are exposed to abiotic stressors like drought, salinity, and heavy metal pollution (Wang et al., 2023a). The present results conform to these accounts, which underscore the necessity for combining microbial and nanotechnology-based strategies for enhancing crop resistance and productivity. In addition to plant productivity and stress resistance, SeNPs also have potential antimicrobial activity, which may be useful in controlling soil-borne diseases and overall crop health (Haggag et al., 2023). Various studies have

indicated that selenium nanoparticles can inhibit the growth of pathogenic fungi and bacteria, thus minimizing the use of chemical pesticides. This double function of SeNPs as growth promoters and microbial threat protectors can further reiterate their significance in sustainable agriculture (Dutta et al., 2023).

Although these preliminary results are encouraging, the semi-field conditions and limited strain number of the study are limitations to be overcome in subsequent studies. One of the limitations in this research is that detailed characterization of the biogenic SeNPs (e.g., TEM, SEM, or particle size distribution) has not been addressed. Future research should incorporate such characterization to ensure nanoparticle stability and properties when under field conditions. Extrapolating the work into field trials, across a broader diversity of bacterial strains, and measuring long-term impacts on soil health will provide additional confirmation of this integrated bioinoculant–nanoparticle strategy's practical scalability.

4. Conclusion

In general, this research provides novel evidence to sustainable agriculture since the selenium-resistant PGPB together with biogenic SeNPs was capable of enhancing plant growth and stress tolerance synergistically. Such combined bio-based method has the potential to be used in improving the productivity of economically valuable crops as well as minimizing chemical fertilizer reliance. It is suggested that future studies must confirm the observed synergistic effects under multi-location field trials and also incorporate molecular-level experiments to understand the mechanisms of PGPB–SeNPs interaction more clearly.

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Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors.

Conflict of interest

The authors declare that they have no conflicts of interest.

Authors contributions

All authors contributed to the study structure, experiments and design. Nayab Batool performed experiments, analyze and grow plants and record results. Rabia Shahdin wrote up the manuscript, performed statistical analysis. Ayesha Siddiqa wrote up the manuscript, designed methodology and reviewed the manuscript.

Sustainable Development Goals (SDGs)

This study directly addresses SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production) by promoting bio-based, eco-friendly

methods to improve crop productivity and reduce chemical inputs. Additionally, it contributes to SDG 13 (Climate Action) by enhancing plant resilience against environmental stress. Indirectly, the work supports SDG 3 (Good Health and Well-being) by improving the nutritional quality of crops.

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