

## Influence of microbial symbionts and nano zinc on soil chemical and biological properties in strawberry field

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### ABSTRACT

In pursuit of sustainable agricultural advancements, this study was carried out over two consecutive years, examining the effects of interaction among arbuscular mycorrhizal (AM) fungi, selenobacteria and nano zinc on the rhizospheric characteristics. The experiment involved two different levels of AM fungi, three levels of selenobacteria and nano zinc oxide each, replicated thrice in factorial randomized block design, under polyhouse conditions. The highest soil concentrations of zinc and selenium were obtained from the combination of AM fungi, *Stenotrophomonas maltophilia* (selenobacterial strain), and 100 mg/L nano zinc. Additionally, there were elevated microbial counts, including total bacterial count, selenobacterial count, phosphorus-solubilizing bacteria and AM spores. The interactive treatments also had a significant impact on soil enzymes. The AM fungi, *Stenotrophomonas maltophilia*, and 100 mg/L nano zinc together recorded increased enzymatic activities of acid phosphatase (55.47%) and alkaline phosphatase (66.69%) than control. This study offers significant findings for improving agricultural methods, which can significantly improve ensuring food security, thereby boosting the adoption of sustainable farming systems.

**Keywords:** AMF, nano zinc, selenobacteria, soil nutrients and strawberry.

### INTRODUCTION

Sustainable agriculture is increasingly reliant on innovative techniques that enhance crop yields while maintaining soil integrity. Exhausting soil nutrients of arable lands, significantly impedes the plant growth and yield, resulting in considerable annual losses in production and income [Fan et al., 2024]. Additionally, land degradation is further accelerated leading to deterioration of soil structure through erosion, contamination and the over-reliance on chemical fertilizers [Zhang et al., 2020]. However, the introduction of naturally

occurring microorganisms, is a promising approach to improve soil structure and mitigate the negative impacts of depleted soil conditions on crop production [Trivedi et al., 2020].

The integration of AM fungi is thought to be a sustainable way to increase crop productivity. Owing to the symbiotic relationships between AM fungi and plant roots, plants may withstand stressful times caused by drought, thereby increasing agricultural output [Naseer et al., 2024]. AM fungi are the organisms that symbiotically attach themselves to plant roots and are inherently biotrophic. By colonizing radicular cortex tissues,

these organisms form a mutualistic symbiosis. AM fungi encourage soil aggregation, through mycorrhizal mycelia and glomalin, thus enhancing the physical and chemical properties of soils. It also aids in the absorption of water and nutrients by plants. Extraradical hyphae generated during plant and AM fungi interactions promote root growth [Mehmood et al., 2022]. Moreover, the AM fungi can boost soil organic matter levels and stimulate soil enzyme activities, which controls rhizospheric soil conditions and subsequently, plant growth. Mycorrhization-stimulated root exudates contribute to feedback control in the soil-plant system which influences the microbial population and soil fertility [Gamal et al., 2022]. Inoculation with PGPRs (plant growth-promoting rhizobacteria) and mycorrhiza has been proposed by several researchers as a potential agricultural benefit [Jaborova et al., 2021]. As per the study done by Duran et al. [2015], selenobacteria can convert inorganic selenium into elemental selenium (Se) nanospheres (NanoSe) along with other significant organic selenium forms through effective inoculation of rhizospheric bacteria with AM fungi, which aids in plant growth.

The prevalent reliance on synthetic chemical fertilizers, especially macronutrients, can be attributed to the pressures farmers face to satisfy market demands, alongside the significant subsidies provided for these products [Singh et al., 2024]. In order to mitigate world hunger and diminishing natural blanket, there is an urgent need to switch to options that are cost-effective as well as ecologically sustainable. The emergence of nanotechnology has created a significant opportunity for progress in this domain, capturing considerable interest due to its connections with various other scientific disciplines [Baig et al., 2021]. Nanotechnology is typically concerned with the creation, analysis and, utilization of nanoparticles (NPs) dimensionally ranging between 1-100 nm [Rajput et al., 2021]. This reduction in size facilitates catalytic activities owing to its reduced particle size and enhanced surface area which thereby, significantly elevates its absorption capacity to a much higher level affecting the crop performance. By regulating nutrient flow according to crop needs, nano-enabled fertilizers may be able to mitigate the problem of fertilizer accumulation [Alsuwayyid et al., 2022]. Nanofertilizers can be suggested as a potential solution for enhancing agricultural productivity. They are known to enhance nutrient absorption and availability owing

to the dynamic location specific nutrient delivery mechanisms [Ghazaryan et al., 2024].

Nano fertilizers also mitigate the rapid leaf burning commonly associated with soluble fertilizer formulations [Singh et al., 2023b]. The capacity of nanoparticles to facilitate the movement of natural nutrients in the rhizosphere is contingent upon their distinctive attributes. Zinc nanoparticles are the most widely utilized nano-sized particles in numerous industries, particularly medicine, cosmetics and agriculture [Singh et al., 2023a]. Zinc nanoparticles are being recently employed as an ingredient in nano fertilizers production to boost plant's growth and vigor [Farooq et al., 2023]. Kopittke et al. [2019] found that applying Zn to the leaves can help increase the absorption of Zn through hyphal transport, biochemical changes in the rhizosphere and physiological changes in plants. This can improve the efficiency of the mutualistic relationship between the host plant and AM fungi. In addition, AM fungi are known to possess the capacity to solubilize residual Zn and convert it into exchangeable or organically bound Zn, thereby enhancing its accessibility.

Strawberries (*Fragaria × ananassa*), is a highly sought after fruit crop, widely cultivated worldwide, including higher elevations of tropical and subtropical zones. This popularity is majorly owing to its versatility in consumption in both fresh and various processed products, like jams, juices, ice creams and pastries [Bisht et al., 2024]. Strawberries are a widely recognized and significant fruit in the world's diet because of their good amount of essential nutrients and beneficial compounds. The high levels of vitamin C, phenolic and folate in strawberries make them a significant source of bioactive compounds. It is among the berries that have been researched the most from agronomic, genetic and nutritional standpoints [Chandler et al., 2021]. The production as well as quality of strawberry is affected by variety of environmental factors and agricultural practices. Soil composition and fertility are particularly crucial, as it thrives best in an adequately drained and nutrient rich soil. The soil supporting the strawberry plants acts as the foundational basis for its growth, providing essential nutrients, maintain optimum pH levels along with ensuring proper drainage [Al-Mamun et al., 2021]. Therefore, soil nutrition is central to successful cultivation of strawberry. Soil condition is crucial in determining the growth of the plant, vitality and disease resistance, henceforth,

significantly influencing fruit taste, quality and shelf life [Duralija et al., 2021]. However, the subject is largely unexplored regarding the interaction between nano zinc, AM fungi, and selenobacteria in strawberries. This study examined how the combined application of nano zinc, AM fungi and selenobacteria affected soil physical, chemical, and microbial parameters, as well as enzymatic activity.

## MATERIALS AND METHODS

### Materials used in experiment

A factorial experiment including three factors viz., Arbuscular Mycorrhizal Fungi [AM fungi] applied in two levels [no AM fungi [AM<sub>1</sub>] or control and AM fungi [AM<sub>2</sub>]], three levels of selenobacteria [no selenobacteria (SeBac<sub>1</sub>), *Stenotrophomonas maltophilia* (SeBac<sub>2</sub>) and *Alcaligenes faecalis* (SeBac<sub>3</sub>)] and another three levels of nano zinc oxide [without nano Zn (n-ZnO<sub>1</sub>), 100 mg/L of nano Zn (n-ZnO<sub>2</sub>), and 200 mg/L nano Zn (n-ZnO<sub>3</sub>)] (Figure 1). The experiment was laid out in a factorial randomized design comprising a total of eighteen treatment combinations, each replicated three times under the polyhouse conditions. AM fungi consisted of a consortium of various mycorrhizal inoculums applied 10g per plant. The mycorrhizal biofertilizer was procured from International Panacea limited, Gurugram, Haryana, India. The two strains of selenobacteria (*Stenotrophomonas maltophilia* and *Alcaligenes faecalis*) used in the study were obtained from the National Bureau of Agriculturally Important Microorganisms (NBAIM), Kushmaur, MauNath Bhanjan, Uttar Pradesh, India. They were cultivated in 200 mL nutrient broth containing sodium selenite [Se (IV), Na<sub>2</sub>SeO<sub>3</sub>] resulting in a concentration of 1–2 × 10<sup>9</sup> colony-forming units per milliliter [Duran et al., 2013]. The rhizoinoculants were applied three times (at the time of transplanting, 30 and 45 days after transplanting), while the foliar application of nano zinc was done twice (30 and 60 days after transplanting). The nano zinc oxide (ZnO) applied in this study was obtained as a white nanoparticulate powder, with its crystalline nature verified through X-ray diffraction analysis. The material possessed an average particle diameter not exceeding 100 nm and contained 79.1–81.5% elemental zinc, with a specific surface area ranging from 15 to 25 m<sup>2</sup> g<sup>-1</sup>. ZnO nanoparticles are classified

as metal oxide nanomaterials and exhibit n-type semiconductor behavior, characterized by a direct band gap of 3.37 eV.

### Cultivation of strawberry

The experiment was performed on a day neutral strawberry cv. Sweet Charlie during the second fortnight of October. The study was conducted over two consecutive growing seasons (2021–22 and 2022–23) under similar agro-climatic conditions. The data recorded from both years were pooled and analyzed together to improve statistical reliability and interpretation. The runners were transplanted onto raised beds at a spacing of 30 × 60 cm accommodating approximately 55,000 runners per ha. This was performed using the double-row planting method. The initial soil physico-chemical profiling of the experimental site was analyzed prior to the commencement of the study and presented in Table 1.

### Experimental location

The experimental block of the Department of Fruit Science, located within the campus of Dr. YS Parmar University of Horticulture and Forestry in Nauni, Solan, Himachal Pradesh, served as the site for laying the experiment. It was located at an elevation of 1275 meters above mean sea level in a sub-temperate zone positioned geographically at 30°52' N latitude and 77°11' E longitude.

### Soil chemical analysis

A maximum of 1 kg soil sample at a depth of 0–15 cm, from each treatment was collected. The analysis of soil pH and electrical conductivity (EC) was done by measuring them in soil water suspensions at a 1:2 ratio. The soil organic carbon (OC) was determined using wet oxidation methodology [Dane and Topp, 2020].

The available nitrogen content was estimated using the alkaline potassium permanganate method. The available phosphorus content was determined using Olsen's method. The concentration of potassium (K) was measured using a flame photometer using the 1 N neutral ammonium acetate method. The concentration of calcium (Ca) and magnesium (Mg) were also determined using a flame photometer, whereas the concentration of Mg was determined using an atomic absorption spectrophotometer. A spectrophotometer utilizing

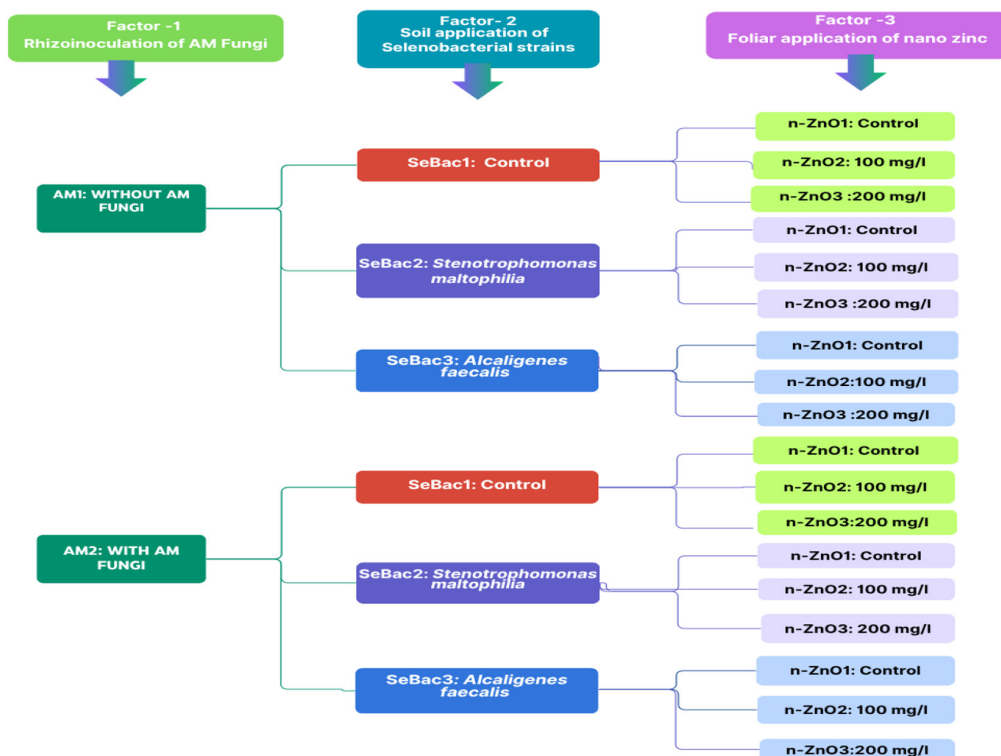


Figure 1. Experimental layout

atomic absorption was used to analyze the concentrations of DTPA-extractable zinc, iron, copper, and manganese. The pH was maintained at  $7.3 \pm 0.05$  using a buffer solution. All the above methods were analyzed as described by Sparks et al., [2020]. However, the Se levels in the collected samples were quantified using inductively coupled plasma-mass spectrophotometer (ICP-MS) [de León et al., 2003].

### Soil microbiological analysis

The total bacterial count was determined using the standard plate counting technique [Lee et al., 2021]. The sterilized nutrient agar medium was inoculated and following 48 hours of incubation at a temperature of  $28 \pm 2$  °C. The microbial count was later quantified as colony forming units per gram (CFU/g) of soil. As described by Ghosh et al., (2008), the serial dilutions of the soil sample in use, lead to isolation of endophytes, tested on 2 mM sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) containing nutrient agar plates. The specimens were incubated for 24–48 hours at a temperature of 37 °C. The bacterial colonies isolate with red colonies were identified as selenobacteria (Trivedi et al., 2020). Phosphorus solubilizing bacteria were detected by observing the development of colonies with a yellow zone

Table 1. The physico-chemical properties of soil prior to the commencement of experiment

Sr No.	Soil properties (soil depth:0–30 cm)	Content
1	pH	6.60
2	EC ( $\text{dSm}^{-1}$ )	0.20
3	OC (%)	0.40
4	N (mg/kg)	157.19
5	P (mg/kg)	16.84
6	K (mg/kg)	142.95

and yellowish fluorescence on dark violet/pinkish medium after the bacterial culture was incubated for 48–72 hours at 37 °C, as described by Hii et al., [2020]. However, the abundance of AM fungi spores in the soil was assessed using the wet sieving and decanting technique [Fuzi et al., 2015].

### Soil enzymatic content

The activities of acidic and alkaline phosphatases were assessed spectrophotometrically at a wavelength of 420 nm. 1 gram of freshly collected soil was incubated using 0.2 milliliters of toluene, buffer solution (4 mL; pH 6 for acid phosphatase and pH 11 for alkaline phosphatase), and p-nitrophenyl phosphate (1 mL concentration

of 0.05 mol l<sup>-1</sup>) at 37 °C for 1 hour. Post incubation, the enzymatic activity was estimated based on p-nitrophenol release [Wang et al., 2017].

### Statistical analysis

The collected data for each parameter was evaluated using analysis of variance (ANOVA) with the assistance of MS-Excel and GRAPES (General Rshiny Based Analysis Platform Empowered by Statistics), version 1.0.0. A Duncan’s multiple range test (DMRT) with a significance level of 5% was employed after the combined ANOVA indicated a statistically significant difference.

## RESULTS AND DISCUSSION

The ANOVA revealed significant effects ( $p < 0.05$ ) of arbuscular mycorrhizal fungi (AM), selenium bacteria (SeB), zinc oxide nanoparticles (ZnO), and their interactions on various soil physicochemical, micronutrient, and microbial/enzymatic properties.

### Effect of AM fungi, selenobacteria and nano zinc on soil characteristics

The experimental data presented in Tables 2 and 3 represents the effect of AM fungi, nano zinc and selenobacterial strains on several soil

parameters. The study revealed that the application of AM fungi positively affected the soil characteristics impacting an improved soil macro and micro nutrient content. The soil micro flora was also significantly improved with AM fungi. The rhizospheric application of selenobacterial strains also impacted the soil quality significantly. The selenobacterial strain *Stenotrophomonas maltophilia* was found to affect significantly the soil parameters including the enhanced available selenium nutrient and selenobacterial content of the soil. The comparative effects of both the selenobacterial strains, it was inferred that SeBac<sub>2</sub> showed better results with regards to soil parameters to the other strain SeBac<sub>3</sub> and control. The foliar spraying with nano zinc resulted in significant changes in the soil nutrient properties. The nutrient content of the soil was significantly improved with the nano zinc applied at the rate of 200 mg/L compared to the other dosage of 100 mg/L and control. The interaction of AM fungi and selenobacteria had affected majority of the soil characteristics majorly the microbial population and the associated enzymatic activity of the soil. The AM fungi and nano zinc interaction was also observed to have significant effects on the nutrient profile and the microbial flora of soil. The interactive effect of selenobacteria and nano zinc was also found to have significantly impacted the soil health status. However, the interaction

**Table 2.** Effect of AM fungi, selenobacteria and nano zinc on soil chemical properties

Factor	pH	OC	EC	N	P	K	Ca	Mg	Fe	Cu
AMF										
Control (AM <sub>1</sub> )	6.64 b	0.46b	0.26a	164.88b	17.71b	145.10b	4.38b	1.70b	64.95b	8.66b
With (AM <sub>2</sub> )	6.76 a	0.61a	0.24b	166.18a	20.45a	159.26a	5.72a	2.07a	68.77a	9.85a
LSD (p <0.5)		0.008	0.003	5.727	0.466	3.692	0.081	0.029	0.845	0.127
Selenobacteria										
Without (SeBac <sub>1</sub> )	6.75a	0.54a	0.26a	170.71b	18.53c	145.35b	5.06b	1.87c	65.30b	8.43c
Strain 1 (SeBac <sub>2</sub> )	6.61b	0.53b	0.25b	169.85a	19.55a	156.08a	5.02c	1.92a	69.43a	10.12a
Strain 2 (SeBac <sub>3</sub> )	6.55c	0.53b	0.25b	168.80c	19.24b	155.11a	5.07a	1.88b	65.85b	9.21b
LSD (p <0.5)	0.124	0.009	0.003		0.57	4.521	0.099	0.036	1.035	0.155
Nano Zinc										
Without (n-ZnO <sub>1</sub> )	6.63b	0.52b	0.25b	168.03b	18.80b	150.13c	4.97c	1.85c	66.36b	8.61c
100 mg/L (n-ZnO <sub>2</sub> )	6.67b	0.53a	0.24c	170.87a	19.33a	152.03b	5.08b	1.89b	67.71a	9.35b
200 mg/L (n-ZnO <sub>3</sub> )	6.81a	0.53a	0.26a	172.71a	19.08b	154.38a	5.09a	1.91a	66.50a	9.79a
LSD (p <0.5)	0.124	0.009	0.003	7.014	0.57	4.521		0.036	1.035	0.155

**Note:** OC – soil organic carbon (%), EC – soil electrical conductivity (dS/m), N – available nitrogen (mg/kg), P – available phosphorus (mg/kg), K – available potassium (mg/kg), Ca – exchangeable calcium [cmol (p<sup>+</sup>) kg<sup>-1</sup>], Mg – exchangeable magnesium [cmol (p<sup>+</sup>) kg<sup>-1</sup>], Fe – soil iron (mg/kg) and Cu – soil copper (mg/kg).

**Table 3.** Effect of AM fungi, selenobacteria and nano zinc on soil chemical properties, soil microbial properties, soil enzymatic properties

Factor	Zn	Mn	Se	TBC	PSB	SeB	AMF	AcP	AIP
AMF									
Control (AM <sub>1</sub> )	15.95b	51.04b	3.55b	60.79b	3.24b	4.85b	196.53b	105.82b	139.05b
With (AM <sub>2</sub> )	16.82a	55.93a	3.72a	64.51a	6.05a	8.31a	542.24a	133.32a	203.28a
LSD (p <0.5)	0.221	0.744	0.056	0.945	0.07	0.782	4.938	1.439	2.196
Selenobacteria									
Without (SeBac <sub>1</sub> )	15.87c	53.49b	3.11b	61.23b	4.72b	1.03c	327.34c	116.47c	161.92c
Strain 1 (SeBac <sub>2</sub> )	16.87a	53.84a	4.34a	64.56a	4.94a	11.73a	411.30a	123.87a	177.04a
Strain 2 (SeBac <sub>3</sub> )	16.12b	53.13c	3.46b	62.18b	4.29c	6.98b	369.52b	118.36b	174.53b
LSD (p <0.5)	0.271		0.069	1.158	0.085	0.958	6.047	1.763	2.689
Nano Zinc									
Without (n-ZnO <sub>1</sub> )	14.53c	51.81b	3.67a	60.44c	3.98b	5.91b	357.57b	112.91c	177.31a
100 mg/L (n-ZnO <sub>2</sub> )	16.99b	54.19a	3.67a	64.55a	5.07a	6.72a	375.63a	123.85a	173.14b
200 mg/L (n-ZnO <sub>3</sub> )	17.64a	54.46a	3.57b	62.98b	4.90a	7.12a	374.96a	121.94b	163.05c
LSD (p <0.5)	0.271	0.911	0.069	1.158	0.085	0.958	6.047	1.763	2.689

**Note:** Zn – Soil zinc (mg/kg), Mn – Soil manganese (mg/kg), Se – Soil selenium (mg/kg), TBC – Total bacterial count ( $\times 10^5$  cfu/g), PSB – Phosphorus solubilizing bacteria ( $\times 10^5$  cfu/g), SeB – Selenobacterial count ( $\times 10^5$  cfu/g), AMF – AM spores count (per 50g soil), AcP – Acid phosphatase enzyme ( $\mu\text{g pNPP/g/ha soil}$ ) and AIP – Alkaline phosphatase enzyme ( $\mu\text{g pNPP/g/ha soil}$ ).

of all the three factor viz., AM fungi, selenobacteria and nano zinc significantly improved the soil chemical indices along with the soil microbial content. The rhizoinoculation of AM<sub>2</sub> along with SeBac<sub>2</sub> and n-ZnO<sub>2</sub> i.e.100 mg/L viz., positively impacted the total bacterial count, phosphorus solubilizing bacteria, total AM fungi spore count and selenobacterial count in the soil. The soil enzymatic activity was also significantly enhanced with this interaction.

#### Interactive effects of AM fungi, selenobacteria and nano zinc on soil chemical properties

The Figure 2–4 illustrates that the interaction among all the factors were effective in improving the soil nutrient profile compared to control. It was evident that the available phosphorus content of the soil was statistically increased with the application of AM fungi and selenobacterial strain - *Stenotrophomonas maltophilia* (Figure 2a). It was evident that by applying AM fungi and SeBac<sub>2</sub> along with nano zinc increased soil potassium levels. The potassium level gradually increased with the application of nano zinc from 0 to 100 mg/L and then it slightly decreased with the dosage of 200 mg/L. The effect of SeBac<sub>3</sub> with the other factors also significantly on par with that of SeBac<sub>2</sub> in influencing the potassium

levels in the soil (Figure 2b). The micronutrient contents of the soil were also impacted with the interaction of all the three factors. The soil iron levels were improved with the soil application of AM<sub>2</sub> and SeBac<sub>2</sub> (Figure 3a). The copper content (10.62 mg/kg) of the soil was significantly enhanced with the application of AM fungi and SeBac<sub>2</sub> along with the application of nano zinc 100mg/L compared to the control. A value at par was recorded with the similar interaction except for the usage of strain 2 viz., *Alcaligenes faecalis* (Figure 3b). The application of AM fungi, *Stenotrophomonas maltophilia* and nano zinc application resulted in higher soil zinc content with 100 mg/L and gradually increased up to 51.37 percent with dose of 200 mg/L compared to the control (Figure 4a). Figure 4b clearly illustrates significant interactive effect of rhizoinoculated AM<sub>2</sub>, SeBac<sub>2</sub> and n-ZnO<sub>2</sub>, that resulted in elevated levels of selenium content of the soil. The soil selenium content was increased by 60.72 percent over the control.

#### Interactive effect of AM fungi, selenobacteria and nano zinc on soil microbial properties

The soil micro flora is indicative of the rhizospheric health which is directly corresponds to the healthy plant growth and nutrient rich produce.

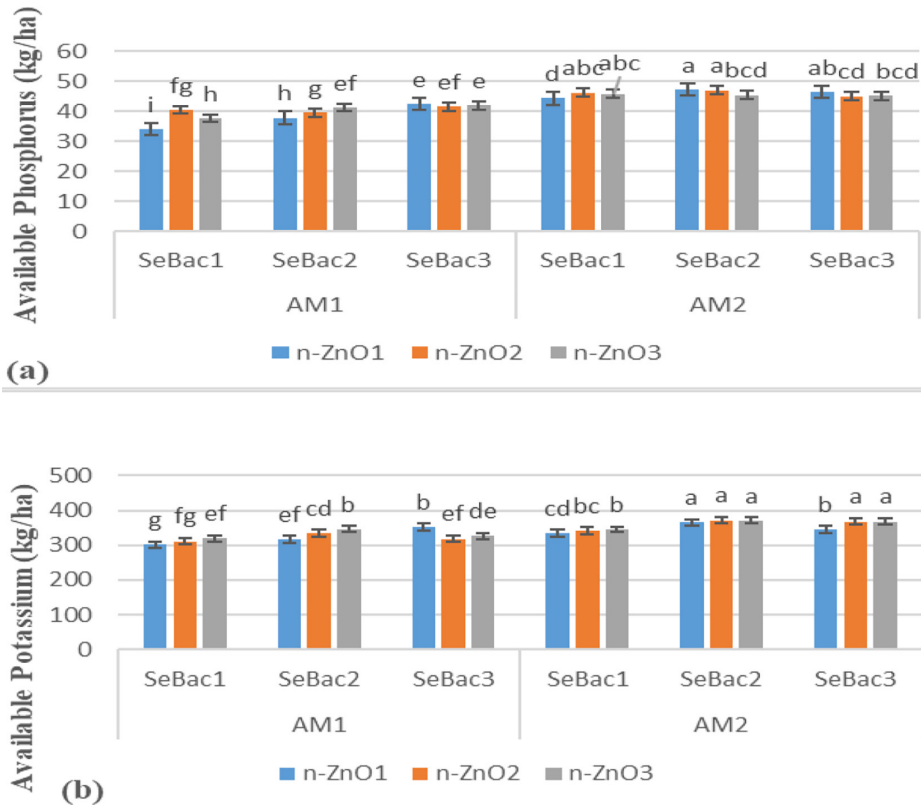


Figure 2. Interactive effects of AM fungi, selenobacteria and nano zinc on: (a) soil phosphorus (mg/kg) content, (b) soil potassium (mg/kg)

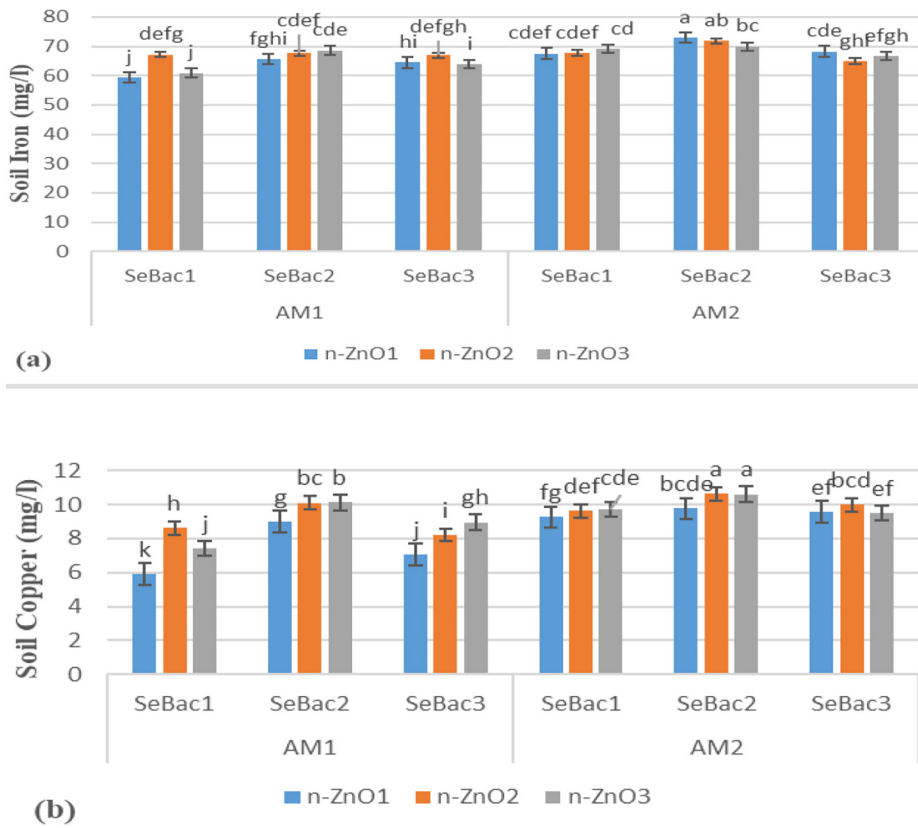
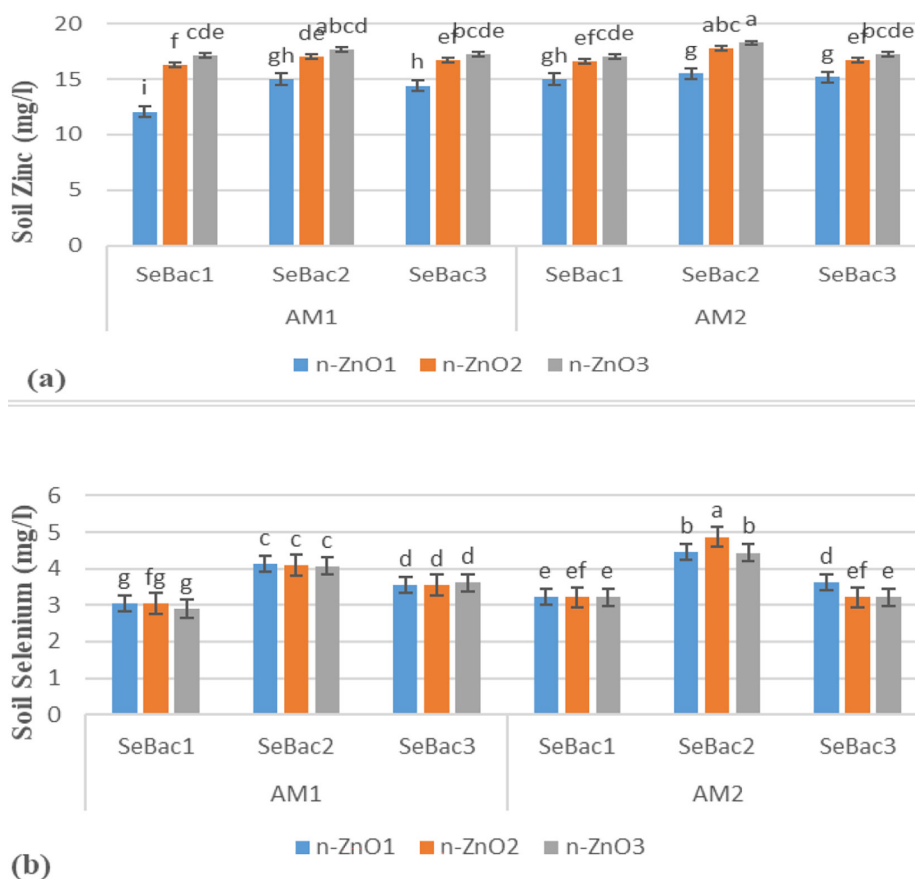


Figure 3. Interactive effects of AM fungi, selenobacteria and nano zinc on: (a) soil iron (mg/kg), (b) soil copper (mg/kg)



**Figure 4.** Interactive effects of AM fungi, selenobacteria and nano zinc on: (a) soil zinc (mg/kg), (b) soil selenium (mg/kg)

It was evident that the rhizoinoculation of AM<sub>2</sub>, SeBac<sub>2</sub> and n-ZnO<sub>2</sub> at 100 mg/L showed significant results in improving the soil microbial content than the control. This interaction recorded a significant increase in total bacterial count ( $70.44 \times 10^5$  cfu/g), selenobacterial count ( $16.00 \times 10^5$  cfu/g), phosphorus solubilizing bacteria ( $7.22 \times 10^5$  cfu/g) and AM spore count (644.07 per 50 g of soil) (Figure 5a-d and 6).

#### Interactive effect of AM fungi, selenobacteria and nano zinc on soil enzymatic activity

The soil enzymes are crucial to ensuring soil fertility, quality and health. Figure 7a shows that the interactive effect of AM fungi, *Stenotrophomonas maltophilia* and nano zinc application (100 mg/L) significantly contributed to an increase in soil enzymatic activities. The acid phosphatase enzyme levels was increased in the soil by 55.47 percent compared to control treatment (Figure 7a). A similar pattern was observed for the presence of soil alkaline phosphatase enzyme. The interaction above marked a significant

increase in the levels of the enzyme by 66.69 percent in comparison to the control (Figure 7b).

#### Principal component analysis (PCA)

In order to evaluate the combinational effects of nano zinc, AMF and two different strains of selenobacteria affecting rhizospheric characteristics along with plant nutrient uptake (Figure 8), the PCA was utilized. The biplots depict the PCA results on the basis of correlation matrix. Every variable is depicted by an arrow, the length of which represents the degree of its contribution to a particular principal component. The degree of correlation amongst the corresponding variables is represented by the angular distance between the arrows- smaller angles denote stronger correlation, while larger angles suggest weaker correlation. In the present analysis, the first (PC1) and second (PC2) principal components accounted for 61.40% and 13.60% of the variations, respectively. The results indicated that almost all the variables had positive contribution as with respect to Dim1, the arrow length of variables

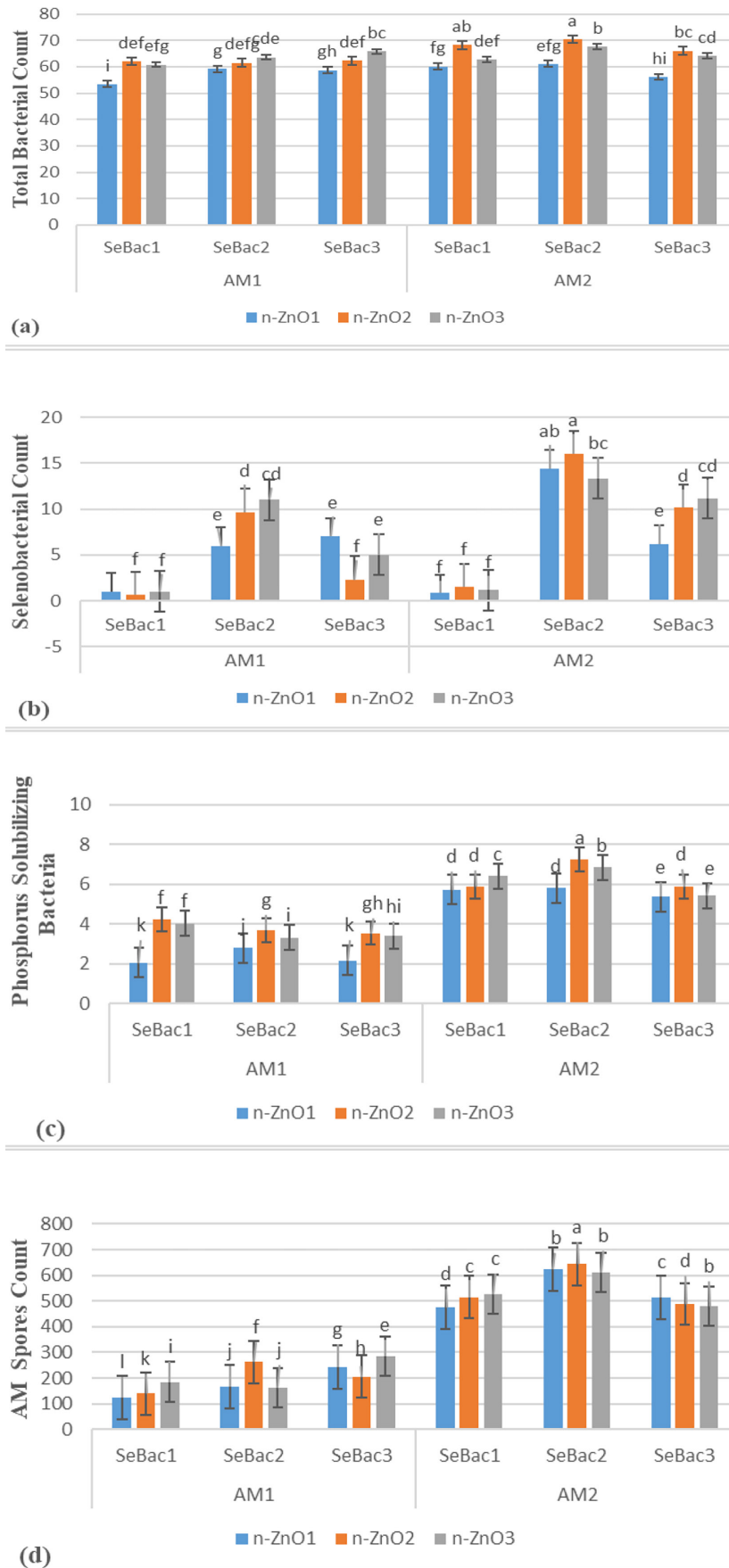
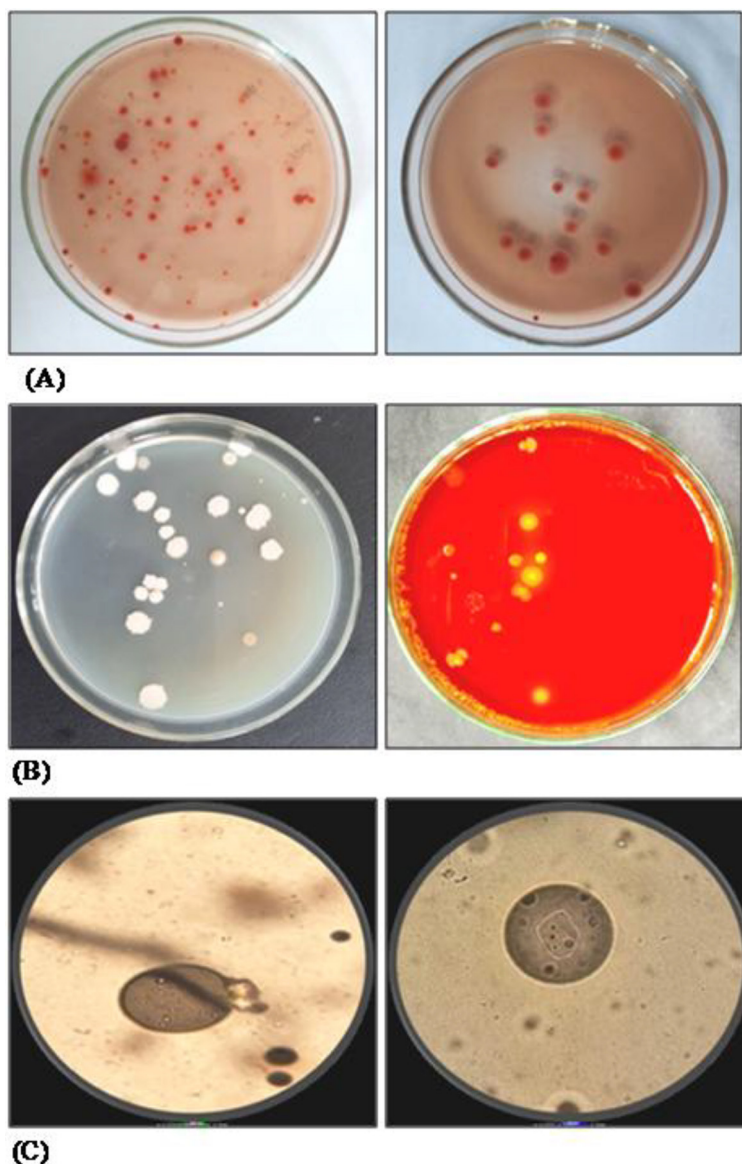


Figure 5. Interactive effects of AM fungi, selenobacteria and nano zinc on (a) total bacterial count, (b) selenobacterial, (c) phosphorus solubilizing bacteria, (d) AM spore count

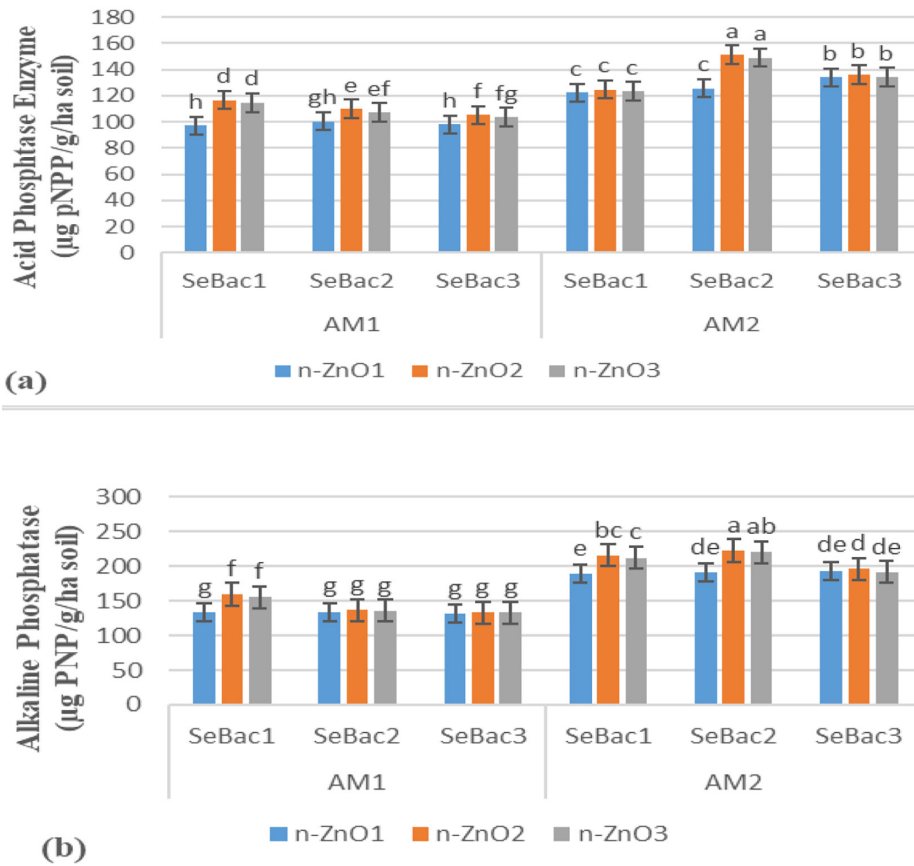


**Figure 6.** (A) Selenobacterial strains in strawberry field (*Stenotrophomonas maltophilia*, *Alcaligenes faecalis*, respectively), (B) total bacterial count and phosphorus solubilizing bacteria, respectively, (C) arbuscular mycorrhizal fungi spores in strawberry field

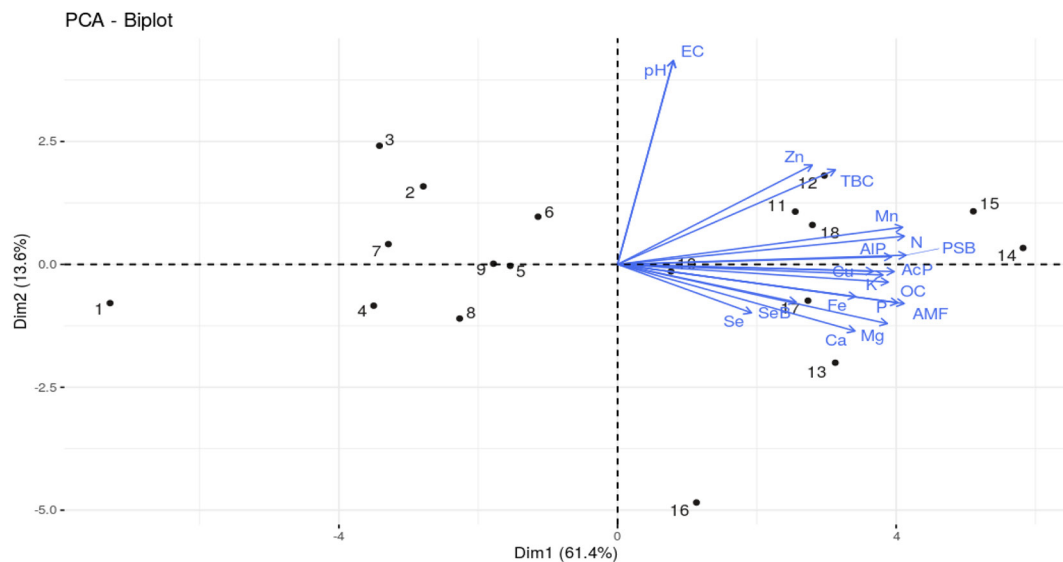
are longer except for Selenium content in soil. Similarly, as the angles are relatively very smaller between different treatment variables except for soil pH and electrical conductivity, representing greater correlation degree among variables. Thus, it is clearly evident that the treatment combination of dual rhizoinoculation of AMF and *Stenotrophomonas maltophilia* alongwith nano zinc oxide at 100 ppm was influential in impacting the overall rhizospheric characteristics. The control treatment with no inputs showed a negative correlation with the Dim1.

The findings regarding soil nutrient content demonstrated that the rhizo-application of AM fungi had a strong impact on various soil

parameters, including soil pH, organic carbon, and several essential nutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, copper, manganese, zinc and selenium. The results of this study regarding the soil pH and organic carbon are in accordance with the findings of Cheng et al. [2012], which showed that the presence of AM fungi caused a significant increase in pH and soil organic carbon in the rhizosphere compared with the absence of AM fungi. AM fungi have been known for their ability of mobilizing phosphorus efficiently. Consequently, these mutually beneficial organisms are identified as fundamental microbial agents of soil that aid in the formation of biogeochemical cycles,



**Figure 7.** Interactive effects of AM fungi, selenobacteria and nano zinc on: (a) acid phosphatase enzyme ( $\mu\text{g pNPP/g/ha soil}$ ), (b) alkaline phosphatase ( $\mu\text{g PNP/g/ha soil}$ )



**Figure 8.** Principal component analysis biplot on the basis of correlation matrix applied to soil chemical properties, soil microbiological properties and soil enzymes (acidic phosphatase and alkaline phosphatase)

specifically those related to phosphorus and nitrogen [Fall et al., 2022]. AM fungi play a crucial role in enhancing the accessibility of phosphorus to the soil. Indeed, it acts as a P activator,

increasing the transformation of P through several chemical reactions, into the forms that can easily be utilized by living organisms through biological interactions [Zhu et al., 2018]. The application of

AM fungi alone resulted in an increase in both acidic phosphatase and alkaline phosphatase levels in the soil. Peng et al., [2020], discovered that soil enzyme viz., alkaline phosphatase content was significantly higher in the group where AM fungi was applied. The sole application of AM fungi led to a noteworthy augmentation in alkaline phosphatase activity, microbial biomass and AM fungi spores in comparison to the control group [Jaborova et al., 2021]. Acid and alkaline phosphatase enzymes facilitate the liberation of inorganic phosphorus from organically bound phosphorus, thereby restoring it to the soil [Prasad 2021]. In their study, Jiang et al. [2023] found that by adding 150 spores/kg soil of AM fungi caused a significant increase of 43.02 µg/kg in the available selenium (Se) content of soil. The utilization of AM fungi resulted in a notable augmentation in the microbiological composition of the soil, as evidenced by the test outcomes. The number of AM fungi spores present in the soil significantly increased as a result of the plants being inoculated with the disease, ranging from 126% to 150%, as reported by Jaborova et al. [2021]. The production of glomalin- a glycoprotein, play a significant role in the formation and stabilizing soil aggregates. Moreover, it has an impact on other microorganisms associated with the mycelia of AM fungi [Prasad, 2021]. The pH and organic carbon content of soil are two essential factors that alter the release of metal ions from nanoparticles, which might have contributed to the abundance of resident microbial populations [Simonin and Richaume, 2015].

It has been observed that selenobacteria favorably influenced the nutrient content of the soil. These bacteria have demonstrated promise in the process of using biological organisms to clean up and enhance soil quality [Eswayah et al., 2019]. It can transform inorganically available Se into elemental nanospheres (NanoSe) and its other essential organic forms [Duran et al., 2013 and Duran et al., 2015]. The plants exposed to selenobacteria displayed noticeably higher levels of micronutrients than plants that did not contain the strain. The phenomena in the current study can be explained in two ways. Firstly, as suggested by Fernandez-Martínez and Charlet [2009] that the attachment of oxides and clay minerals to the surface makes it highly prone to reacting with amino acids and peptides that are secreted by the strains and are eventually released into the soil. Secondly, according to Lee et al. [2011], an additional

possible reason could be secreted extracellular phosphatase, which breaks down insoluble phosphorus in the soil and liberates phosphorus while activating and releasing selenium. A notable increase in enzyme content was noted because of the effect of selenobacteria on soil enzyme levels. The most likely explanation is that the soil contains insoluble phosphorus, which the strain *Stenotrophomonas maltophilia* secretes as an extracellular phosphatase to dissolve. According to Lee et al., [2011], this process triggers phosphorus release, after which Se is activated and released. The interactive effect of AM fungi along with the selenobacterial strain- *Stenotrophomonas maltophilia* was observed to have positively affected the soil enzymatic activity of acid phosphatase which through improved activity of the roots, enhanced the Se absorption [Jiang et al. 2023].

The application of nano-ZnO as a spray on the leaves also affected the chemical properties of soil. Romero-Freire et al. [2017] documented that with the application of ZnO nanoparticles, there was an increase in soil pH. Additionally, the increased number of resident microbes can aid in mineralization and organic acid production, resulting in the neutralization of soil pH. It has been reported that biologically produced chelators cause insoluble forms of Zn to become soluble, resulting in increased soil Zn levels. Furthermore, a correlation had been observed between the rate of application of nano zinc and an increase in the concentration of dissolved Zn. These findings correspond with those reported by Bala et al. [2019]. Alterations in the rhizospheric bacterial community were ascribed to the significant impact of nano ZnO. Bala et al. [2019] documented that the application of ZnO nanoparticles to the leaves led to an increase in the indigenous microbial community which thereby, positively affected the rhizospheric dehydrogenase enzyme activity.

## CONCLUSIONS

The present study indicated that the application of nano Zn at a concentration of 100 ppm on the leaves, along with the rhizoapplication of selenobacteria (*Stenotrophomonas maltophilia*) and AM fungi, can prove to be an efficient interactive treatment for positively impacting the chemical and microbiological properties of soil surrounding the plant roots. They primarily stimulated root activity, thereby enhancing the availability

of macro and micro nutrients, primarily selenium in the rhizospheric zone. The AM fungi and *Stenotrophomonas maltophilia* augmented the soil enzymatic activities viz., acid and alkaline phosphatase enzyme. These findings provide a fresh insight into the microbiological processes that drive mobilization of different mineral nutrients in the soil, thereby enhancing soil health. Thus, paving the way for the innovative cultivational practices for horticultural produces with higher nutritional values.

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