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# Synergistic effect of foliar co-application of *Bradyrhizobium japonicum* and Nanoparticles on Growth, and Productivity of Soybean (Glycine max L.) in Sandy Soil

## Nano-*Bradyrhizobium* co-application Enhances Soybean in Sandy Soil

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## Abstract:

Many strategies, including the application of nanoparticles (NPs), that boost the growth and productivity of plant in sandy soil. The impacts of co-application of N, P, Zn, Fe nanoparticles (NPs) and *Bradyrhizobium japonicumon* on soybean were investigated by pot experiment conducted in Faculty of Agriculture, Cairo University. The plants were exposed to 14 treatments (T1-T14) of 100ppm of N,P NPs and 40ppm of Fe, Zn NPs in exchange with *B. japonicum* inoculation and control (T0 non-treated plants*). B. japonicum* was grown at various concentration of NPs but the high growth rate was observed at 100ppm of N,P NPs and 40ppm of Fe, Zn NPs. And the optimum temperature and pH for its growth was (30-35) and (6.5-7), respectively. *B. japonicum* showed high nitrogenase activity, exopolysaccharides, IAA, (P, Fe, Zn mobilization) with 100ppm of N,P NPs and 40ppm of Fe, Zn NPs. The co-application of *B. japonicum* and nanomaterials were essential for boosting the viability of macro and micro elements in sandy soil, leaves and seed. The co-application of N, P, Zn, Fe NPs and *B. japonicum* (T7) showed an increase in Chlorophyll (3.82-9.54mg/g FW), total Carbohydrate (15.54- 20.67%) and Leaf area (115.41-122.74 cm2) in leaves. Furthermore, P, Zn, Fe NPs, and *B. japonicum* (T11) recorded the highest oil content(17.63%), where T7 increased protein content (32.43%) and the weight of 100 seed (8.07gm) compared to T0 t. The obtained results provide strong evidence that the foliar application of NPs improves the symbiotic performance between *B. japonicum* and the soybean plant.

**Key words:** Sandy soil, soybean, Nanoparticles, Rhizobium, Nutrient viability, Seed quality.

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visualization, supervision, data analysis. All authors agreed for submission of manuscript to the journal.

## 1. Introduction

Soybeans (*Glycine max*) grow well in a variety of climates, temperate tropical, and sub-tropical regions. It is essential crop, has nutritional value and known as “poor man’s meat,” **Tafida *et al*.(2022**). In Egypt, soybean farming has emerged as a practical agricultural solution; face the threats such as import reliance, food security and economic diversity. Soybean farmingintroduces to Egypt in the twentieth century and become a major crop, especially in the Valley and Nile Delta areas. It has several and important uses either asspanning animal feed, human food products or in industrial applications **FAO (2021)**. Egypt is regarded as the leading producer of soybeans in North Africa due to its climate, which includes ideal temperature ranges, exposure to sunlight, and irrigation techniques **Mahmoud *et al*. (2024).** In the year 2022, Egypt's soybean yield was 2.92 t/ha. Globally, Egypt is arranged as the seventh-largest importer of soybeans coming after China, but in 2019 it became the highest importer reaching about 4257.30271 thousand tons for 1663.182 million USD **FAO (2021)**. Despite prosperity of soybean cultivation in Egypt, water scarcity, and limited availability of cultivated land andpest management are challenges facing soybean cultivation and productivity **Salama *et al*. (2023)**.

Sandy soil, is characterized by its large poresand low organic matter content,poses a particular challenge to cropsproductivity due to their quick water drainage and poor nutrient-holding capacities**(Ayman *et al.*, 2020) and (Musei *et al*., 2024)** .Sandy soils are widely distributed around the world, covering approximately 900 million hectaresand in Egypt it represents more than 70% of total area **FAO (2021)**. In dry lands, where the sandy soils are widespread, the anticipated variability and change in climate are expected to aggravate food security problems for farmers who depend on these regions. Sandy lands reclamation through strategies that alleviate water and nutrientpercolation has been developed for increasing crop productivity and assure food security (**Aoda *et al*., 2021).**

Nanotechnology is a recent technique for the enhancement of the agricultural sector because it introduces new ways to alleviate different soil stresses and boost crop yield **(Singh *et al*., 2023 and Vardumyan *et al*., 2024)**.Nano-fertilizer is an important part of modern agricultural techniques, enhancing productivity **Zahra *et al*. (2022)**,able to penetrate the

epidermis with great efficiency, regulate nutrient uptake, reduce wastes, and improvement of nutrients utilization **Ninama, *et al.*(2023) and Mahmoud *et al.* (2023)**.Nanotechnology is one of powerful strategy for improving the properties of sandy soil and protecting plants from its poverty of due to the very high surface area of nanomaterials, surface charges, and their morphological characteristics**(Ayman *et al.,* 2020) and (Dawood, 2021).(Figure 1)**showed a graphical illustration the role of nanomaterials in improving plant growth in sandy soil.

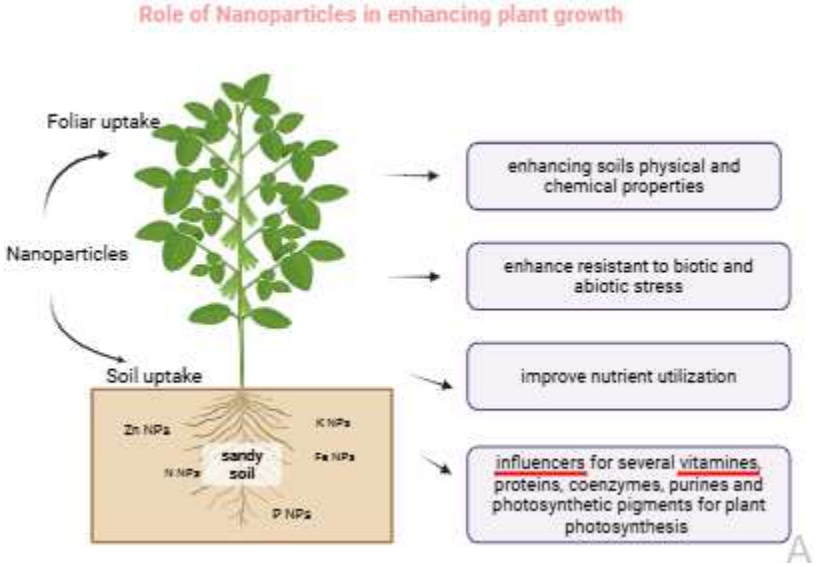


**Figure (1): showing a graphical illustration the role of nanomaterials in improving plant growth in sandy soil design by Biorender.com.**

The over using and applying of chemical fertilizers in agriculture to enhance crop yields and soil productivity, it creates environmental problems, including reduced soil fertility and biodiversity**Khalid*et al.* (2023) and (Mahmoud and Swaefy 2020).** To overcome on the harmful impacts of synthetic fertilizers, different techniques are recently being used to boost plant growth and yield, such as fallowing and green manure cultivation (**Ma *et al.*, 2021**), **Shang *et al.*(2021)** and promotion of biofertilizers (**Castro *et al.*, 2020**) and (**Mahmoud *et al.* 2017**). Rhizobia, are Gram-negative bacteria have a positive impact on crop productivity and nutrient utilization such as boosting phosphorus and iron uptake,that stimulate crop growth, reduce and prevent the harm of pests and plant pathogens, and stimulate the growth of benefited microbes (**Ahemad and Kibret, 2014**). Enhancing nitrogen fixation by bacteria in soybean plant is essential to the sustainable boosting of agriculture by rhizobia biofertilization. The species of rhizobia isolated from soybean plants act as biofertilizers that enhancing N-fixation and decrease

emissions (**Soumare *et al*., 2020**). Rhizobia produce plant growth regulating hormones like gibberellin that has essential role in the growth of nodules in legume plants (**Nett *et al*., 2022).** Additionally, rhizobia are eco-friendly environment, bio-fertilization and has been reported to increase the productivity of most plants, like soybeans (**Kolapo *et al*., 2025**). Investigating novel techniques for the cooperative interactions between microbes, nanomaterials, and plants may assist in reducing various stressors and promote plant development. This three-way relationship improves plant health and provides a viable method for guaranteeing agricultural approaches are sustainable (**Campos *et al*., 2023**).

Upon previous introduction the current investigation was planned to determine the effects of different concentrations of N, P, Zn, Fe nanoparticles (NPs) on *Bradyrhizobium japonicum* growth and its plant growth regulating traits and studied the effect of foliar co-application of N, P, Zn, Fe nanoparticles (NPs) and *Bradyrhizobium japonicum* inoculum on soybean growth and the productivity of this important legume in sandy soil.

## 2. Material and Methods

### 2.1. Effect of the synthesized nanoparticles on rhizobium growth

To observe the effect of various nanomaterials on the growth of rhizobia, the strain of rhizobia (*Bradyrhizobium japonicum*) was obtained from biofertilizer production unit, Soils, Water and Environment Research Institute, Agriculture Research Center (ARC), Giza, Egypt. *Rhizobium* was cultivated in yeast mannitol broth medium **(Vincent, 1970)** only as control, and with N nanoparticles at concentrations 100, 150, 200 ppm, P NPs at concentrations 50, 100 and 150ppm and also with each Fe, Zn nanoparticles at concentrations 35, 40 and 50 ppm then incubated at 28°C for 72 hours under shaking conditions and the total viable count and the size of colony was determined by agar plate method.

### 2.2. Effect of different temperature on rhizobia growth in the presence of nanoparticles

Test tubes was contained with 5 ml yeast mannitol broth medium mixed with previous nanoparticles at its concentrations inoculated with 1 ml of rhizobia $4\times 10^{8}$cfu / mL and incubated at different degrees of temperature 15, 25, 30, 35, 40, 45 $^{\circ}$C in triplicates for three days then the rhizobia growth was visualized.

### 2.3. Effect of different pH values on rhizobial growth in the presence of nanoparticles

Test tubes containing 5ml of yeast mannitol broth medium and the pH was adjusted as 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 pH values and after their sterilization they were inoculated with *Bradyrhizobium japonicum* 4× $10^8$cfu / mL culture with the previous nanoparticles with their concentrations in triplicates and incubated at 30°C for three days then the rhizobia growth was observed.

**2.4. Detection of plant growth regulating (PGP) activities of rhizobium**

The quantitative assay of IAA was carried out in broth YM medium as reported by **Brick *et al*. (1991).** The YM broth medium was distributed in flasks 100ml capacity each contain 50ml supplemented with l-tryptophane (0.5%), then inoculated with 1 ml of rhizobia 4× $10^8$cfu / mL and the flasks were treated with different concentration of four NPs and incubated for three days at 30°C for three days in shaking incubator at 120 r/min. After incubation, the culture medium undergo to centrifugation at 4000r/min for 20min and 1 ml of supernatant was mixed with 2ml of Salkowsky reagent (2% 0.5 M FeCl3 in 35% perchloric acid) and incubated in dark conditions for 30 min. Pink or red color absorbance was measured at 530nm. The amount of IAA in supernatant was estimated using a standard curve of pure IAA. Rhizobium strain was screened to estimate EPS production according **to (Damery and Alexander, 1969)**. *Bradyrhizobium japonicum* allowed to grow on 50ml YM liquid medium with 1% glucose with various concentrations of N, P, Zn and Fe nanoparticles and inoculated with 1 ml of rhizobia 4× $10^8$cfu / mL and incubated in rotary shaker at 200rpm for 3 days. The culture medium was centrifuged at 3500 rpm for 30 minutes after that the supernatant was combained with ow volume of acetone .Consequently, centrifugation was carried out at 3500rpm for 30, the crude of polysaccharides was washed with dis. Water and acetone alternately and filtered by filter paper and weighted after drying at 105° C overnight . A simple method for estimating nitrogenase activity by *Bradyrhizobium japonicum* through reduction of acetylene on agar plates with 2 to 2.5 mM glutamine as sole nitrogen is described by **Wilcockson and Werner (1978)**. The ammonium phosphate molybdate blue color method was used to quantitatively estimation of phosphate solubilization. The blue color absorbance was read at 660nm using KH2PO4 as standard and the amount of soluble phosphate were expressed as mg/l over control **(Jackson, 1973).**Quantitative estimation of zinc solubilizing potential of rhizobium on YM broth medium with 0.1% ZnO based on method described by **Francis *et al*. (1988).** *Bradyrhizobium japonicum*

was grown on 5ml of YM broth medium supplemented with various concentrations of N, P, Zn and Fe nanoparticles and incubated for 72 hours at 30°C. After three days the culture medium was centrifuged 3000 rpm for 20 min then 0.5 mL of supernatant mixed with 0.5 mL of Chromo Azurol Sulfonate (CAS) solution and 10 µL of sulfosalicylic acid solution to make final volume and kept at room temperature for 2hr, the absorbance was calculated at 630nm using UV-spectrophotometer **Bandemer and Shamble (1944).** All experiments were carried out in triplicates with the same treatments. Tukey test ($p \leq 0.05$) was used to compare the treatment means using the high range statistical domain (HSD).

**2.5. Synthesis of Nitrogen, Phosphorus, Iron and Zinc nanomaterials**

The top to bottom molecular chemical technique was used to synthesis nanomaterials. From its precursor, Nitrogen, phosphorus, Iron and Zinc nanomaterials were prepared and synthesized (ammonium nitrate salt $NH_4NO_3$, urea ($CO(NH_2)_2$), Calcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$), magnetite ($Fe_3O_4$) and zinc chloride ($ZnCl_2$)) the used precursor and other reagents were bought from Sigma Chemical Company (Saint-Louis, Missouri, US).

**2.5.1. Synthesis of Nitrogen, Phosphorus nanoparticles**

Nitrogen and phosphorus nanomaterials (Figures 2 a and b) were prepared according to **(Mahmoud and Swaefy, 2020)** with modified method by using chitosan solution as a carrier to polymerize methacrylic acid, for 18 hours it was allowed to coated at room temperature in a buffer solution, in two-steps approaches. Firstly, for 5 hours we dissolved 0.23 g of chitosan in a methacrylic acid aqueous solution (0.5%, v/v) with magnetically stirring. Secondly, with stirring continuously and individually, 0.2 mmol of mix ammonium nitrate salt $NH_4NO_3$ and urea ($CO(NH_2)_2$) (1:1), and 0.4 mmol of Calcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$), were added to the solution separately tile the solution get clear. Subsequently the polymerization was carried out at 45˚C for 7 hours with magnetically stirring, this result in formation of a nanomaterial solution that was consequently exposed to pressure 1.5 psi for 72 hours, discontinuously 7 hours /day, and after that it was centrifuged at 1200 rpm for 35 minutes, and then cooled for two hrs. in an ice bath.

**2.5.2. Synthesis of Zinc and Iron nanoparticles**

Zinc nanomaterial (Figure 2 c) was synthesized by using an aqueous solution of zinc chloride ($ZnCl_2$). Solution of sodium hydroxide was slowly added drope by drope in a molar ratio of 1:2 with strongly mixing for 8 hours. The obtained precipitate was filtered and washed

thoroughly with deionized water, then stirred with water/toluene system by high speed stirrer, then rewashed with deionized water only for 2 hours, after that the precipitate was centrifuged for 40 minutes at 1000 rpm, and then allowed to cool in an ice bath for two hrs. The precipitate get dried at 100 °C by using an oven, subsequently exposed to pressure 1.5 psi for 72 hours discontinuously (7 hours / day) (**Daneshvar *et al.*, 2007) and (Abdel Wahab *et al.*,2020).** As for Nano iron (Figure 2 d) was synthesized with a little modified method from magnetite ($Fe_3O_4$) of Fe3+ and Fe2+ at a molar ratio of 3:2 by reduction–precipitation in aqueous ammonia (0.3 mol/L) and coated tetramethyl ammonium hydroxide under strong stirring for 2 hours by magnetically stirring, subsequently the precipitate was separated by using magnetic filtration with a permanent magnet, then it was washed with dis. water tile reached to a neutral pH, afterward centrifuged at 1400 rpm for 25 minutes according to **Qu *et al*. (1999) and Abdel Wahab and Sahar (2018).**

The size and state of nano-particles were noticed straight by Transmission Electron Microscopy (TEM) utilizing an electron speed increase voltage of 60 kV. The TEM tests were ready by dropping a couple of drops of the arrangement on a carbon-covered copper framework, the work were finished in TEM labs (Histo. Unit) in Fac. of Agric. Cairo College Research Park (FA-CURP).

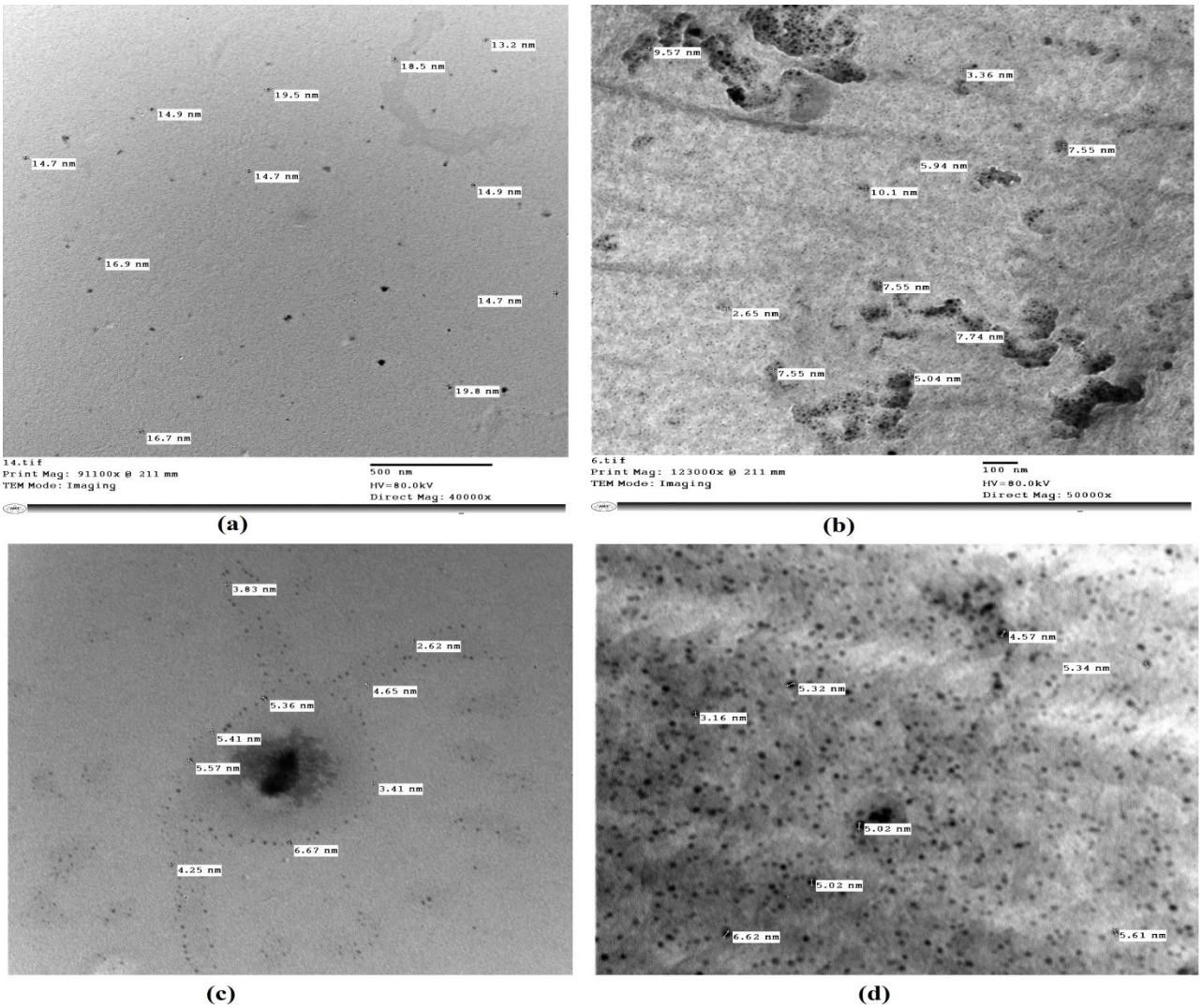


**Figure (2): Showing Nano particles** a)Nano-nitrogen, b) Nano-Phosphorus, c) Nano-Zinc, d) Nano-Iron.

## 2.6. Pot experiment

The current investigation was carried out at the greenhouse of Agricultural Research Center, Giza, Egypt within two summer growing seasons 2023 and 2024. In this experiment the Soybean cultivar was Giza 111, that obtained from Field Crops Research Institute, Agricultural Research Center, Giza, A.R. Egypt.

This experiment was designed to investigate the impact of four elements of nanofertilizers: nitrogen (NF), Phosphorus (PF), Iron (FeF) and Zinc (ZF), on morphology, yield components and chemical composition of soybean *Glycine max* (L.), plants were grown in sandy soil Table (1) obtained from Cairo-Alexandria Desert Road.

**Table 1: The physical and chemical characteristics of sandy soil**

| **Physical properties** | | **Cations (meq / l)** | | **Anion (meq / l)** | |
|---|---|---|---|---|---|
| **Sand (%)** | 90.5 | $Ca^{++}$ | 4.16 | $CO_3^{=}$ | 0 |
| **Silt (%)** | 6.50 | $Mg^{++}$ | 1.52 | $HCO_3^{-}$ | 0.54 |
| **Clay (%)** | 3.0 | $Na^{+}$ | 3.24 | $Cl^{-}$ | 3.31 |
| **Texture grade** | Sandy | $K^{+}$ | 0.21 | $SO_4^{=}$ | 5.28 |
| **pH (1:2.5)** | 7.8 | Micronutrients (ppm) | | | |
| **EC (dS/m )** | 1.37 | Fe | 2.4 | | |
| **Total-N (%)** | 0.03 | Zn | 0.4 | | |
| **Organic-C (%)** | 0.38 | Cu | 0.3 | | |

### 2.6.1. Plant materials and experimental procedures

Seeds of soybean were sown on May 12, 2023, in plastic pots (40 cm in diameter), that filled with sandy soil after washed it three times with water and then mixed with compost Table (2) at a rate of 5 tons / fadden for all treatments.

**Table 2. The physical and chemical analysis of the compost applied**

| **Property** | **Value** |
|---|---|
| Moisture content (%) | 25 |
| PH (1:5) | 7.6 |
| EC (1: 5 extract) $dSm^{-1}$ | 3.2 |
| Organic-C (%) | 30.1 |
| Organic matter (%) | 51.7 |
| Total-N (%) | 1.4 |
| Total-K (%) | 1.27 |
| C/N ratio | 20:1 |
| Total-P (%) | 1.2 |
| Fe (ppm) | 1018 |
| Zn (ppm) | 279 |
| Total content of Bacteria ($cfu.g^{-1}$) | $2.3 \times 10^7$ |
| Phosphate dissolving Bacteria ($cfu.g^{-1}$) | $2.6 \times 10^6$ |
| Weed seeds | 0 |

The experiment design was coordinated in a randomized complete block design (RCBD) with triplicates. Each block had fifteen different nano-fertilizer treatments Table (3) in randomized

distribution. Each replicate had three pots; contains three holes for each pot, after the start of germination, thin out and leaved 2 plants in each hole.

**Table 3: Treatments used in pot experiment**

| No. | Treatments | Concentration |
|---|---|---|
| **1** | T0 (Control) | (as a recommended) |
| **2** | T1 (NF)+rhizo. | 100ppmN |
| **3** | T2 (NF & PF) +rhizo. | 100ppmN +100ppmP |
| **4** | T3 (NF &ZnF) +rhizo. | (100ppmN)+ (40ppmZn) |
| **5** | T4 (NF &FeF) +rhizo. | (100ppmN)+ (40ppmFe) |
| **6** | T5 (NF & PF &ZnF) +rhizo. | (100ppmN)+(100ppmP)+ (40ppmZn) |
| **7** | T6 (NF & PF &FeF) +rhizo. | (100ppmN)+(100ppmP) +(40ppmFe) |
| **8** | T7 (NF & PF &ZnF&FeF) +rhizo. | (100ppmN)+(100ppmP)+(40ppmZn)+ (40ppmFe) |
| **9** | T8 (PF) +rhizo. | (100ppmP) |
| **10** | T9 (PF &ZnF) +rhizo. | (100ppmP)+ (40ppmZn) |
| **11** | T10 (PF &FeF) +rhizo. | (100ppmP) +(40ppmFe) |
| **12** | T11 (PF &ZnF&FeF) +rhizo. | (100ppmP)+(40ppmZn)+ (40ppmFe) |
| **13** | T12 (ZnF) +rhizo. | (40ppmZn) |
| **14** | T13 (ZnF&FeF) +rhizo. | (40ppmZn)+ (40ppmFe) |
| **15** | T14 (FeF) +rhizo. | (40ppmFe) |

**NF: nitrogen as nanofertilizers, PF: Phosphorus as nanofertilizers, FeF: Iron as nanofertilizers and ZF: Zinc as nanofertilizers, rhizo.: rhizobium.**

### 2.6.2. Recommended additions

For the all treatments phosphorus and potassium fertilizers were applied as a basal dressing. Each pot delivered the following basic treatments: 5.6 g/ pot (225 kg/ fadden), two weeks before sowing calcium super phosphate (15.5% $P_2O_5$) was added, then two weeks after sowing 1.5 g/ pot (60 kg/ fadden) potassium sulphate (48% $K_2O$) was added and repeated after one month later, mineral nitrogenous fertilizer (MN): Ammonium sulphate (20.6%N) was added at (20 units of nitrogen / fadden) It was added at the time of planting, and after 30 days, 20 units of nitrogen were added,

### 2.7. Statistical analysis

The obtained results represented average of two seasons was undergrown to proper analysis of variance as revealed by **Snedecor and Cochran (1980)**. At 5% the least significant differences (L.S.D.) were calculated for each tested character under various assigned treatments.

## 3. Results

### 3.1. Effect of the synthesized nanoparticles on rhizobium growth.

The viable total count of rhizobia showed different growth response to different concentrations of nanoparticles and also different range of colony size Table (4), rhizobia recorded high growth rate and large colony size with N, P NPs and Fe, Zn NPs at concentration 100 ppm, 40ppm respectively whileN, P NPs showing low growth rate at concentration 200 and 150 ppm, respectively.

**Table (4): Effect of the synthesized nanoparticles on *B. japonicum* growth.**

| **Parameters** | *B. japonicum*&**N**conc. (ppm) | | | *B. japonicum*&**P** conc. (ppm) | | | *B.japonicum*&**Fe** conc. (ppm) | | | *B.japonicum*&**Zn** conc. (ppm) | | | *B. japonicum* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 150 | 200 | 50 | 100 | 150 | 35 | 40 | 50 | 35 | 40 | 50 | - |
| Colonies number | 8-10 | 3-5 | 2 | 2-3 | 7-8 | 1-2 | 4-5 | 6-7 | 1-2 | 2-3 | 4-6 | 1-2 | 5-6 |
| Colonies diameter mm | 2-3 | 1-1.5 | 0.8 | 1.5-2 | 3-4 | 0.8-0.7 | 1.5-2 | 2-3 | 0.6-0.7 | 1-1.5 | 3-3.5 | 0.8-0.9 | 1.5-2 |
| Growth rate | +++ | ++ | + | + | +++ | + | ++ | ++ | + | + | ++ | + | ++ |

**Conc.: concentration +++: strong growth ++: moderate growth +: poor growth**

The current results showed that rhizobia strain was thermotolerant, it grow well at 30°C as well as 37°C with 100ppm concentration of N and P NPs and 40ppm concentration of Fe and Zn NPs, it also able to grow below and above this degrees Table (5), moreover rhizobia strain it can grow at wide range of pH it recorded high growth rate under slightly acidic condition at concentration 100and 150ppm, 50and 100ppm, 35 and 40ppm for N, P, Fe and Zn, respectively Table (6).

**Table (5): Effect of different temperature on *B. japonicum* growth in the presence of nanoparticles.**

| **Measurement** | **N** conc. (ppm) | | | **P** conc. (ppm) | | | **Fe** conc. (ppm) | | | **Zn** conc. (ppm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Temperature C°** | 100 | 150 | 200 | 50 | 100 | 150 | 35 | 40 | 50 | 35 | 40 | 50 |
| 15 | + | + | - | + | + | - | + | + | - | + | + | - |
| 25 | + | + | - | + | + | - | + | + | - | + | + | - |
| 30 | +++ | + | + | + | +++ | + | + | ++ | + | + | ++ | + |
| 35 | ++ | + | - | + | ++ | - | + | ++ | - | + | ++ | - |
| 40 | + | + | - | + | + | - | + | + | - | + | + | - |
| 45 | + | + | - | - | + | - | - | - | - | - | - | - |

**Conc.: concentration+++: strong growth ++: moderate growth +: poor growth- : no growth**

**Table (6): Effect of different pH values on *B. japonicum* growth in the presence of nanoparticles.**

| Measurement pH | N **conc. (ppm)** | | | P **conc. (ppm)** | | | Fe **conc. (ppm)** | | | Zn **conc. (ppm)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 150 | 200 | 50 | 100 | 150 | 35 | 40 | 50 | 35 | 40 | 50 |
| 5.5 | - | - | - | - | + | - | + | + | - | + | + | - |
| 6 | + | + | - | + | + | - | ++ | ++ | - | ++ | ++ | - |
| 6.5 | +++ | + | + | ++ | +++ | + | ++ | ++ | + | ++ | ++ | + |
| 7 | ++ | ++ | - | ++ | ++ | - | ++ | ++ | - | ++ | ++ | - |
| 8 | + | + | - | + | + | - | + | + | - | + | + | - |
| 8.5 | + | + | - | + | + | - | + | + | - | + | + | - |
| 9 | + | + | - | - | - | - | - | - | - | - | + | - |

### 3.2. Plant growth promoting (PGP) traits of *B. japonicum.*

The results showed varying levels of PGPtraits wereproduced by *B. japonicum* at different concentrations of NPs. The quantitative assaying of IAA in broth YM culture supplemented with (0.5%) L- Tryptophan, showed variation in the quantity of IAA produced by *B. japonicum*. The highest quantity of IAA (12.01µg/ml) was recorded at 40 ppm of Zn NPs, followed by (11.26µg/ml) for 100ppm of N NPs, where produced low concentration of IAA 1.886 µg/ml at 50ppm of Fe (Figure 3a).

As shown in (Figure 3b), the maximum amount of EPS production was 31.5 µg $ml^{-1}$ followed by 27.5 µg $ml^{-1}$ observed at 100ppm of N and P NPs respectively, on the other hand the amount of EPS by *B. japonicum* individually was 20.8 µg $ml^{-1}$, on the other hand the lowest concentration of EPS obtained at 50ppm of Fe5.9 µg $ml^{-1}$.

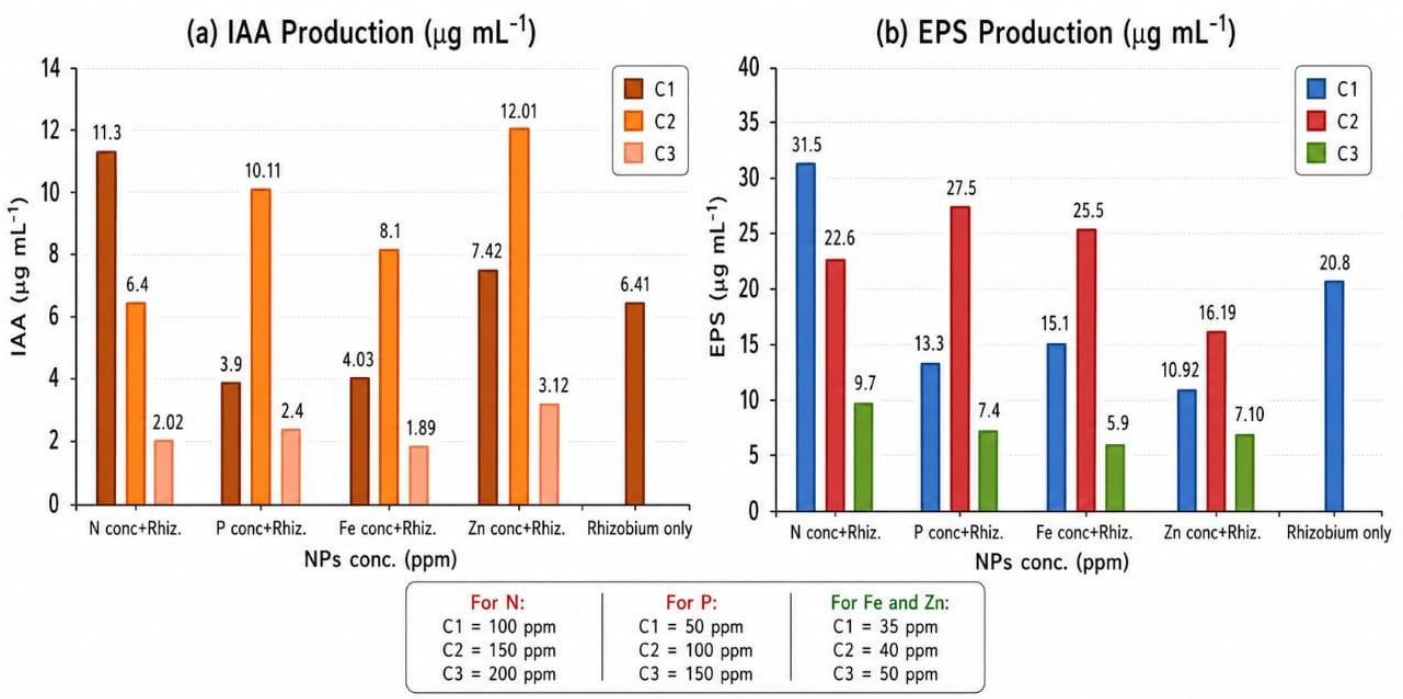


**Figure (3)**: **Plant growth promoting (PGP) traits of *B. japonicum*, a) IAA Production b) exopolysaccharides (EPS) production at different concentrations of NPs.**

Table (7) showing that the effect of various concentrations of NPs on nitrogenase activity and minerals solubilization by *B. japonicum*. The nitrogenase activity was more efficient at low concentration of N NP 6.49 (µmol C2H4 $h^{-1}$), whereas *B. japonicum* only recorded 4.02(µmol C2H4 h-1). The highest phosphate solubilization by *Bradyrhizobium japonicum* was 22.64ppm recorded at 100ppm P NPs while the rhizobium strain only recorded 15.02, furthermore 40ppm conc. of Fe and Zn recorded high phosphate solubilization rather than the other two concentrations 35 and 50ppm of them. Additionally, the high concentration of nano-Nitrogen resulted low P solubilization 5.77ppm. The efficiency of zinc solubilization by *B. japonicum*was found to vary relying on the concentration of nanomaterials, the highest zinc solubilization was 0.73ppm determined at 40ppm Zn NPs, while the lowest Zn solubilization was 0.08ppm at 150ppm P NPs. The *Bradyrhizobium* strain treated with 100 ppm of Fe NPs showed high Fe solubilization 0.93ppm compared to *Bradyrhizobium* only where it recorded 0.44ppm while 150ppm of P NPs observed the lowest Fe solubilization 0.11ppm.

**Table (7): Showing that the effect of different concentrations of NPs on minerals solubilization by *B. japonicum*.**

| Treatments | Concentrations | Mean of Nitrogenase activity (µmol $C_2H_4$ $h^{-1}$) | Mean of P conc.(ppm) | Mean of Zn conc.(ppm) | Mean of Fe conc.(ppm) |
|---|---|---|---|---|---|
| *B. japonicum* &**N** conc. (ppm) | 100 | 6.49a | 17.81b | 0.55cd | 0.79bc |
| | 150 | 4.07e | 13.92g | 0.31g | 0.42e |
| | 200 | 1.86i | 5.77m | 0.1h | 0.13h |
| *B. japonicum* &**P** conc. (ppm) | 50 | 3.55f | 14.29f | 0.32g | 0.23g |
| | 100 | 5.94c | 22.64a | 0.51de | 0.63d |
| | 150 | 2.03i | 10.07j | 0.08h | 0.11h |
| *B.japonicum* &**Fe** conc. (ppm) | 35 | 2.75h | 11.73h | 0.49e | 0.75c |
| | 40 | 4.97d | 15.88d | 0.61b | 0.93a |
| | 50 | 1.51j | 8.86k | 0.1h | 0.42e |
| *B.japonicum* & **Zn** conc. (ppm) | 35 | 3.29g | 10.92i | 0.57bc | 0.64d |
| | 40 | 6.21b | 16.19c | 0.73a | 0.81b |
| | 50 | 1.20k | 7.1l | 0.28g | 0.33f |
| *B. japonicm* | - | 4.02e | 15.02e | 0.38f | 0.44e |
| | LSD | 0.194612 | 0.209294 | 0.041982 | 0.044005 |

### 3.3. Effect of co-application of N, P (100ppm) NPs and of Fe, Zn (40ppm) NPs and *B. japonicum* on soil's minerals concentration.

As shown in Table (8), the co-applying *B. japonicum* and NPs in sandy soil improved significantly the soil fertility, physical, chemical, and biological characteristics of soil. The data obviously show that the co-applying *B. japonicum* and NPs significantly enhanced the soil's minerals concentration (N, K, Fe and Zn) compared to the control treatment. N, K, Fe and Zn content in soil showed significant increase with T7 47.69, 187.60, 13.27 and6.49ppm, respectively compared to T0 40.17, 180.65, 8.12 and4.26ppm, respectively, where T11showed high significant in P content 9.85ppm whereas T7 come in the second order 9.38ppm.The increase of N, P, K, Fe and Zn contents in soil associated with N fixation and P, Zn, Fe mobilization in the rhizosphere by *B. japonicum*or may supplied by NPs . The viability of minerals is maybe due to the exopolysaccharides produced by *B. japonicum* that improve the texture of soil, water and nutrients retention by sandy soil.

**Table (8): Effect of foliar co-application of N, P Fe, Zn NPs and *B. japonicum* on soil's minerals concentration. (Results represented the average of two seasons).**

| Treatments | N ppm | P ppm | k ppm | Fe ppm | Zn ppm |
|---|---|---|---|---|---|
| **T0** | 40.17b | 6.42ba | 180.65a | 8.12b | 4.26a |
| **T1** | 32.30d | 3.39c | 168.38c | 7.63b | 2.39b |
| **T2** | 37.79c | 7.72a | 170.58b | 7.90b | 2.77b |
| **T3** | 38.29cb | 5.33b | 175.93b | 7.95b | 4.52a |
| **T4** | 38.97cb | 4.94b | 177.14b | 8.12b | 3.13b |
| **T5** | 40.78b | 8.43a | 179.38a | 7.33b | 4.79a |
| **T6** | 42.86b | 8.51a | 181.61a | 11.47a | 4.33a |
| **T7** | 47.69a | 9.38a | 187.60a | 13.27a | 6.49a |
| **T8** | 26.20e | 7.61a | 166.23c | 7.28b | 2.40b |
| **T9** | 27.47e | 8.22a | 168.56c | 7.10b | 3.53b |
| **T10** | 26.42e | 8.61a | 169.86c | 10.20a | 3.65b |
| **T11** | 39.90b | 9.85a | 160.74d | 12.16a | 6.24a |
| **T12** | 25.20e | 5.41b | 165.83d | 7.18b | 4.62a |
| **T13** | 27.71e | 6.83ba | 173.57b | 10.43a | 5.05a |
| **T14** | 24.55e | 5.44b | 161.20d | 11.85a | 2.13b |

**Results represented the average of two seasons. The values of mean followed by different letters verify significantly by LSD at $p < 0.05$.**

### 3.4. Nutritional status of the leaves

Table (9) displays the impact of Foliar spraying of both nanoparticles and rhizobium on the leaves chemical composition. With NPs and *B. japonicum* treatments, the treated soybean leaves recorded a significant increase in the mounts of all nutritional elements (N, P, K, Fe, Zn and Mn) of soybean leaves compared to control plants. The uppermost increase in nutritional status of the leaves N, K and Zn 6.26, 1.67 and 50.36%, respectively were observed in the plants exposed to T7 , where T1 was less significant in N and Zn 3.79 and 22.7%, respectively, additionally T12 resulted in low K percent 1.33% compared to control . Likewise T11 showed significant increase in P, Fe, and Mn 0.52,70.79 and 23.20 respectively.

**Table (9): Effect of foliar co-application of N, P Fe, Zn NPs and *B. japonicum* on leaves's minerals concentration. (Results represented the average of two seasons).**

| Treatments | N % | P % | k % | Fe % | Zn % | Mn% |
|---|---|---|---|---|---|---|
| **T0** | 4.06b | 0.32c | 1.34d | 40.21d | 25.06c | 15.12c |
| **T1** | 3.79c | 0.32c | 1.38d | 41.24d | 22.70d | 18.07a |

| | | | | | | |
|---|---|---|---|---|---|---|
| **T2** | 3.89c | 0.35c | 1.48c | 47.33c | 24.72cd | 18.13a |
| **T3** | 4.69ab | 0.37c | 1.52b | 46.25c | 44.79b | 20.16a |
| **T4** | 4.67ab | 0.44b | 1.50b | 52.69b | 26.84c | 19.18a |
| **T5** | 4.59b | 0.42b | 1.55b | 48.51c | 46.98a | 20.93a |
| **T6** | 5.61a | 0.45b | 1.53b | 53.10b | 27.45c | 21.33a |
| **T7** | 6.26a | 0.50a | 1.67a | 68.35a | 50.36a | 22.18a |
| **T8** | 4.41b | 0.42b | 1.39d | 40.08d | 26.19c | 18.07a |
| **T9** | 5.16a | 0.40b | 1.43c | 43.12d | 28.74c | 19.15a |
| **T10** | 5.11a | 0.48a | 1.42c | 55.28b | 30.86c | 17.67ab |
| **T11** | 6.16a | 0.52a | 1.55b | 70.19a | 49.81a | 23.20a |
| **T12** | 4.65ab | 0.42b | 1.33d | 41.64d | 45.29a | 16.92b |
| **T13** | 6.08a | 0.44b | 1.37d | 54.20b | 48.29a | 19.05a |
| **T14** | 4.16b | 0.40b | 1.37d | 52.36b | 27.34c | 17.36ab |

**Results represented the average of two seasons. The values of mean followed by different letters verify significantly by LSD at $p < 0.05$.**

### 3.5. Chlorophyll content in leaves

Figure (4) shows the influence of foliar co-application of P&Zn&Fe NPs with *B. japonicum* on chlorophyll content in leaves of soybean plant. With respect to the other treatments, T11 treatment significantly increased chlorophyll content, achieving the highest values 9.54 mg/g FW followed by T7 8.49 mg/g FW, whereas the lowest values were realized with the corresponding plants exposed to T1 3.82 mg/g FW. With regard to the interaction effect of NPs, the most significant improvement in all measured chlorophyll content was observed when plants treated with P&Zn&Fe NPs with *B. japonicum*.

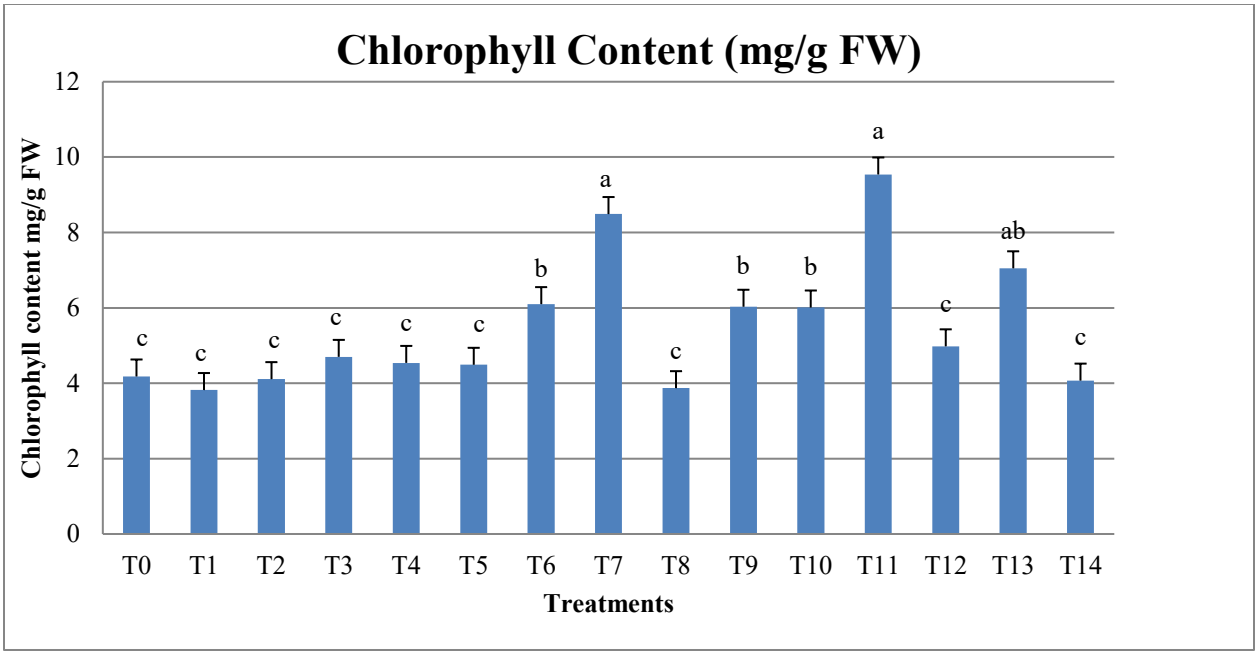


**Figure (4): Effect of foliar co-application of NPs and *B. japonicum* on leaves chlorophyll content. (Results represented the average of two seasons). The significant differences among treatments was indicated by different letters (P<0.05).**

### 3.6. Effect of foliar co-application of NPs and *B. japonicum* on leaves Total Carbohydrates content and Leaf area

The foliar application of NPs and *B. japonicum* enhanced significantly the total carbohydrates of soybean leaves and leaf area (Figure 5, 6). The highest increases in total content of carbohydrates and leaf area of soybean's leaves were recorded for the co-application of NPs and *B. japonicum*, especially with (N, P, Zn, Fe) NPs and *B. japonicum* T7 followed by T11. Such increases were higher than the corresponding ones recorded for the application of other treatments. In this concern, T7 seemed to be the most efficient treatment recording significant increases in total carbohydrate content and leaf area by 20.67% and 122.74 $cm^2$, respectively higher than the non-amended control treatment, on the other hand T1 recorded significant decreases in both total carbohydrate content and leaf area by 15.54% and 115.41 $cm^2$ .

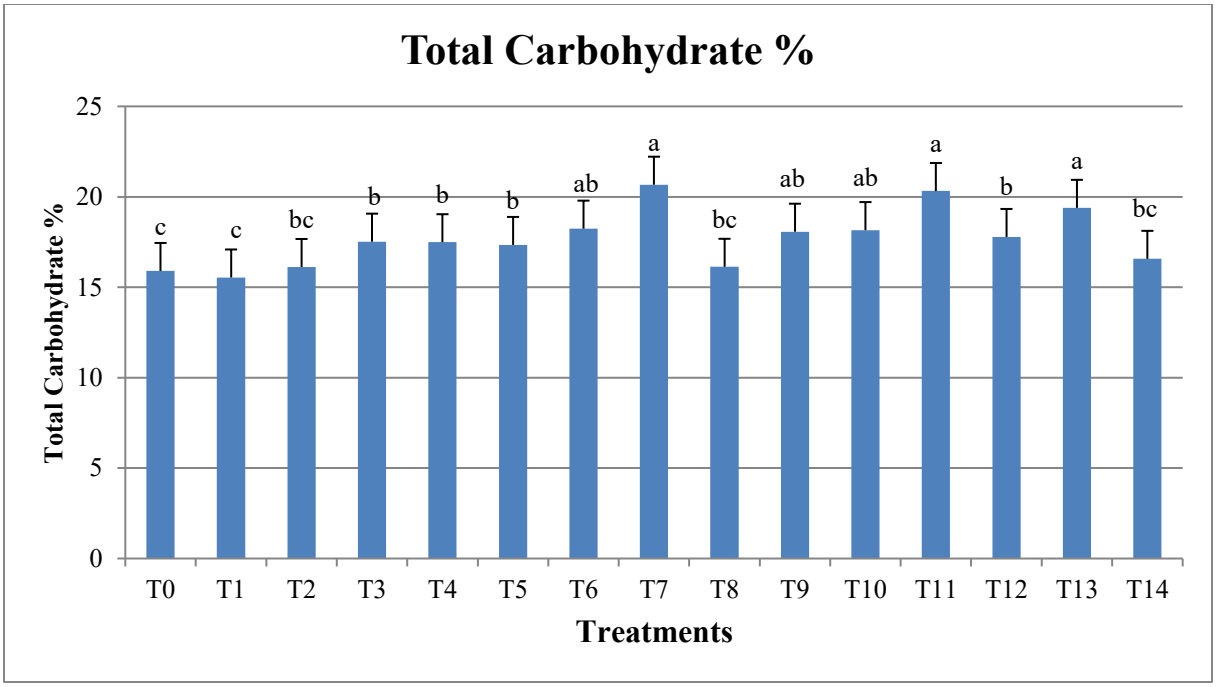


**Figure (5): Effect of foliar co-application of NPs and *B. japonicum* on leaves total carbohydrates. (Results represented the average of two seasons). The significant differences among treatments was indicated by different letters (P<0.05).**

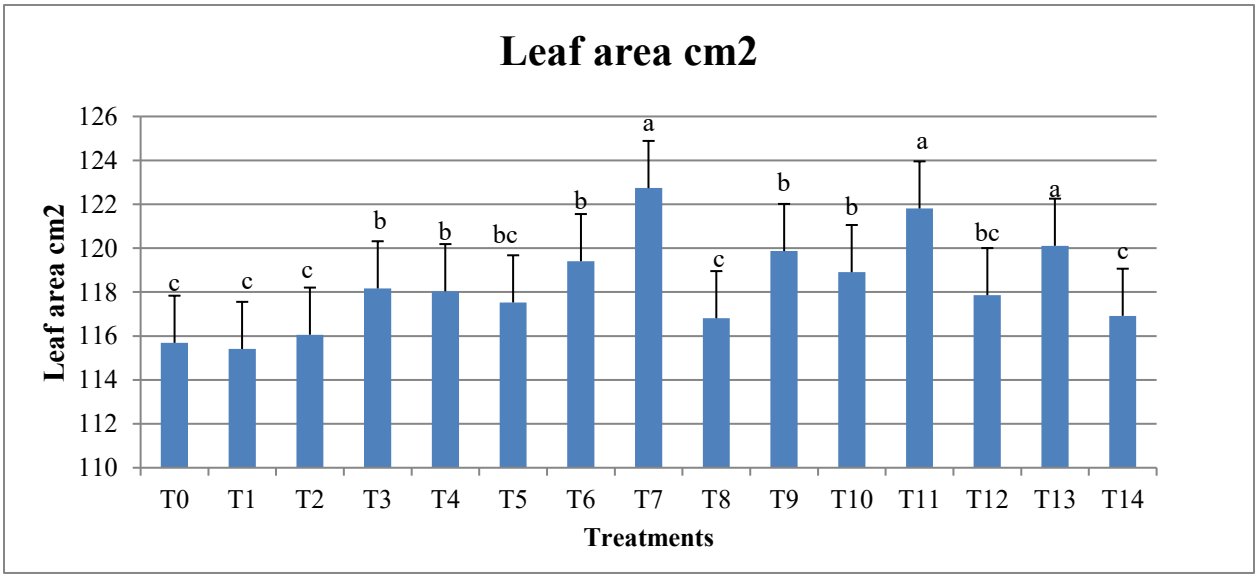


**Figure (6): Effect of co application of NPs and *B. japonicum* on leaf area. (Results represented the average of two seasons). The significant differences among treatments was indicated by different letters (P<0.05).**

### 3.7. Effect of foliar application of various nanoparticles (N&P&Zn&Fe) and *B. japonicum* on yield attributes of soybean in sandy soil.

Table (10) illustrates that the synergistic effect of *B. japonicum* and the nanoparticles (N& P& Zn& Fe) as foliar application have a positive impact on seed's macronutrients (N 6.33%, P 0.42%), micronutrients (Zn 46.87ppm, Fe 110.87ppm), however T10 (P& Fe NPs and *B. japonicum)* was significant increase in K 1.51%. The data illustrate that the highest positive influence on the investigated seed properties was achieved due to the foliar co-application of (N& P& Zn& Fe) nanoparticles and *B. japonicum*. This mean that th e companied NPs and *B. japonicum* enhanced the seed quality of soybean compared to control treatment.

**Table (10): The Effect of various co-application nanomaterials (N&P&Zn&Fe) and B. *japonicum* on yield attributes of soybean in sandy soil.**

| Treatments | N% | P% | k% | Zn ppm | Fe ppm |
|---|---|---|---|---|---|
| **T0** | 3.90b | 0.31b | 1.42b | 40.84b | 80.97c |
| **T1** | 4.27ab | 0.30b | 1.42b | 43.98ab | 89.18b |
| **T2** | 4.33ab | 0.33b | 1.45ab | 43.99ab | 93.50b |
| **T3** | 5.16a | 0.36ab | 1.45ab | 45.93a | 102.24a |
| **T4** | 5.12a | 0.35b | 1.45ab | 44.54ab | 101.61a |
| **T5** | 5.09a | 0.38ab | 1.47a | 44.40ab | 101.25a |
| **T6** | 5.10a | 0.37ab | 1.47a | 44.34ab | 100.46a |
| **T7** | 6.33a | 0.42a | 1.37b | 46.87a | 110.87a |
| **T8** | 3.87b | 0.22c | 1.41b | 40.90b | 81.79c |
| **T9** | 4.92ab | 0.36ab | 1.44ab | 44.21ab | 97.39ab |
| **T10** | 4.92ab | 0.33b | 1.51a | 44.09ab | 97.35ab |
| **T11** | 6.24a | 0.40a | 1.47a | 46.70a | 108.98a |
| **T12** | 5.15a | 0.39a | 1.40b | 45.63a | 102.71a |
| **T13** | 6.15a | 0.41a | 1.41b | 45.64a | 106.12a |
| **T14** | 3.91b | 0.29b | 1.41b | 40.88b | 82.47c |

**Results represented the average of two seasons. The values of mean followed by different letters verify significantly by LSD at $p < 0.05$.**

The nutritional value of soybean (Seed protein and oil content) and the weight of 100 seed showed notable enhancement in response to the foliar spray of (N& P& Zn& Fe) NPs and *B. japonicum*. The plants treated with T7 significantly increase the oil percent 19.12%, the amount of protein in seed (32.43% ) and also recorded the heaviest weight 100 seed (8.07gm) compared to T0 (control).Despite T7 recorded highly significant on seed quality but T11

(P&Zn&Fe and *B. japonicum* ) was significant increase in oil percent 18.93%, the amount of protein in seed (38.97% ) and also recorded increase in the weight 100 seed (10.27gm) compared to T0 (control) (Figure7). Our above results proved that *B. japonicum*has the ability to fix nitrogen, P Zn and Fe solubilization, therefore these results have positively reflect on the percent of N, P, Zn and Fe in seed.

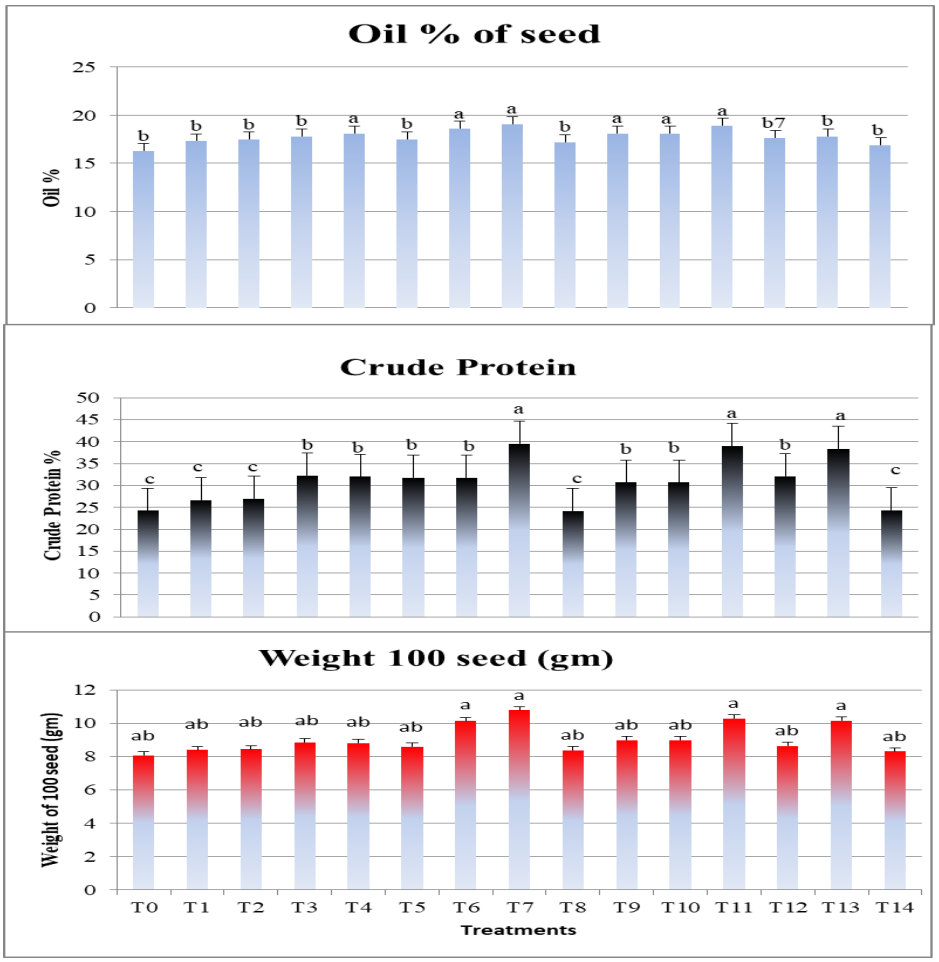


**Figure (7).yield attributes of soybean as affected by co application nanoparticles (N&P&Zn&Fe) and *B. japonicum.* The significant differences among treatments was indicated by different letters (P<0.05).**

## 4. Discussion

Although, nanoparticles exert inhibitory activity against most microorganisms, but rhizobia able to grow strongly in the presence of N, P, Fe and Zn NPs at 100 and 40 ppm respectively compared to control (rhizobia only). This may be due to nanoparticals would increase the viability of nutrients for rhizobia and provide a conducive habitat for its

establishment or even acting as a source of nutrients for bacteria. According to **Dhas *et al.* (2014),** bacteria probably accommodate to grow in the presence of nanomaterials or transfer them to another form to lower their toxicity during the lag phase of bacterial growth curve.

From the results it was concluded that nanoparticles induce rhizobium to tolerate a wide range of pH and temperature this explained by Niño ***et al.*** **(2019)** bacteria have ability to conjure numerous response mechanisms to adapt with nanoparticles in their growth habitats such as efflux pumps (membrane-bound transport proteins), electrostatic repulsion, altered morphology, formation of biofilm, extracellular matrices, transfer of genes, mutations, and metabolic responses.

On the other hand, nanoparticles have a positively effect on plant growth regulating characteristics, including production of growth hormones, siderophores and fixation of nitrogen by regulating the expression of genes *amoA1*and *amoC2* that responsible for nitrification **(Amanda *et al*., 2021**), this result is harmony with **(Dimkpa *et al.*,2012b)** who found that the plant growth-promoting bacterium *Pseudomonas chlororaphis* showed increasing in IAA synthesis at 200 mg/L of CuO NPs, where the lowest concentration of IAA investigated at (1.89µg/ml) for 50 ppm of Fe NPs. In this study, the obtained results were significantly lower than that recorded by **Seidu*et al.*** **(2025)** where the amount of IAA produced by *Bradyrhizobium sp.* ranged from 54.97 ± 3.21–108 ± 12.10 µg/ml. The amount of EPS produced by *B. japonicum* contrast to **Ali and Hussein (2021)** who found that *Rhizobial* strains 3612, 1799 and 3629 showed EPS production 4.13, 3.62 and 3.54 g/L respectively, this due to the presence of NPs in growth culture at suitable concentration induced EPS production by *B. japonicum*. Our concerns were in line with the prior findings of **Khalid *et al.*** **(2020)**, who reported that different species of *rhizobium* are efficient in phosphorus solubilization.

These findings investigated that *B. japonicum* in the presences of NPs at certain concentration increase minerals solubilization, this support **Welch and Vandevivere's (1994)** hypothesis that naturally occurring polymers can affect mineral dissolution by acting as attachment structures to the mineral or rock surface. Moreover, the fresh microbial EPS increases the rate of feldspar dissolution, probably by forming complexes with framework ions in solution this confirmed by **Meena *et al.*** **(2014)**. **Alharbi *et al.*** **(2023)** reported that the co-application of nanoparticles and plant growth promoting rhizobacteria was generally found to increase the

content of mineral nutrient in rhizosphere rather than separately applied of nanoparticles or PGPR.

The foliar spray of NPs and *B. japonicum* resulted in increment in macro and micronutrient in soil and different plant parts like leaves and seed of soybean plant increase minerals solubilization and plant nutrient- uptake. This result is harmony **(De Souza *et al*., 2021)** who demonstrated that the contents of Fe in soil and plant parts such as nodules, roots and shoots was increased by ($Fe_3O_4$ nanoparticales and *Rhizobium* sp. treatments) reveals that they enhance plants uptake of Fe from soil. Additionally (**Liu *et al*., 2022; Verma *et al*., 2023a**) reported that the content of soil nitrate was increased as a result of co-applying of nanomaterials and plant growth regulating rhizobacteria in comparison to a single dose of nitrogen. Morever, NPs and PGPR boosted content of organic carbon, phosphorus, and nitrogen availability more than normal plants. Furthermore, NPs boosted rhizobial exopolysaccharides production, which plays a crucial role in enhancing structure of soil and retention of water, ultimately benefiting plant growth. EPS act as"glue," binding soil particles together, improving soil aggregation and reducing water and wind erosion. Interestingly, **Raliya *et al*. (2014)** added ZnO nanomaterials to culture medium of *Bacillus subtilis* strain JCT1 for enhancement EPS production that was improved aggregation in 33–83% in sandy soil, hence induce nutrient availability.

As the total minerals content increase in treated plants this may be regarded to accumulation of significant quantities of these minerals rather than control plants. This improvement in minerals content results from enhancement of nutrient uptake that promoted by introduced NPs and *Rhizobium*. The obtained results were close agreement with the previous results of **De Souza *et al*. (2021)** who found an increase in total nitrogen content in shoot (26.5–50.2%) and root (24.1–48.2%) of treated plants compared to control plants due to the effective combination between Fe3O4 NPs and *Rhizobium* inoculation. From this result, nanoparticles and *Rhizobium* sprayed on plant leaves increase in minerals in leaves more than the control this due to integration of *rhizobium* and NPs enhanced plant nutrient- uptake, this is in consistency with **Szopa *et al*. (2022)** who stated that the interaction between microbes and nanoparticle can boost nutrient uptake by plants encapsulated in nanoparticles, especially at detrimental conditions. Similarly, **(De Souza *et al*., 2019)** investigated the synergistic action of NPs and *Rhizobium* lead to an increase in growth parameters of plants and also concentration of mineral content including Ca,

K, P, Fe, and Mn in all plant parts. These results were in similarity **Bashandy *et al*. (2024**) who found that the content of N, P, Zn, and K was high in seeds of faba bean plants due to triple application of microbial inoculation with Rhizobium, seaweed extract, and Zinc Oxide Nanoparticles.

The important role of *B. japonicum* in increase of nutrient solubility in addition to soil and foliar application of nanoparticles that enhance root absorption and pass on nutrient through stomata and allowing plants to receive essential nutrients, hence boosted the activity of the photosynthesis process, resulting in high chlorophyll content in leave. Our result is harmony with **Mahmoud and Sahar (2018) Ding *et al*. (2025)** revealed that the foliar application of combined of Fe and Zn-NPs showed high chlorophyll content (16.6–44.3 %) in wheat more than combination of (Zn), (Fe), and silicon NPs. Accordingly, it can be clarified that the usage of Zn and Fe-NPs in combination aggravated the level Fe and Zn in the chloroplast and therefore under drought conditions the photosynthetic pigments in wheat plants were enhanced. Additionally, **Sheta *et al*. (2024)** documented that the content of carbohydrate in cowpea leaves increased after Zn-NP and Fe-NP applications due to good growth of plant. On the other hand, **Rehman *et al*. (2023)** illustrated that the application of Zn-NP and Fe-NP increased, total phenol, proline, and carbohydrate content in wheat grown in saline soil.

The co-applying of *B. japonicum* and N&P&Zn&Fe showing significantly increase in leaf area in comparison to the other treatments. The obtained results could be explained as stated by **Ahmed *et al*. (2012) and Mahmoud *et al.* (2022)** who reported that Zinc (Zn) is responsible for elongation and enlargement of plant cells by stimulate tryptophan synthesize and its enzymes, that are responsible for biosynthesis of indole acetic acid beside that IAA produced by *B. japonicum* which exhibited more leaf growth.

On the other hand, co-application of NPs with *B. japonicum* boosted the increment seed's protein percent this due to nitrogen availability, both N taken by *B. japonicum* from the air, N provided by NPs and N available in the soil that influence amio acids formation and increase protein formation in seed this result is harmony with **Surtiningsih *et al*. (2009)** who reported that the availability of N from soil and air nitrogen fixation by *Rhizobium* bacteria is initially stacked on the stem and leaves and when the pods are formed, nitrogen is collected into pods, and about (30 - 90%) of N being absorbed into the seeds. Additionally, **Grangah *et al*. (2020)**

stated that nano-fertilizers especially Fe + Zn treatment improved the content of protein in bean seeds. Our results investigated that the co-application of nanoparticles and *B. japonicum* has positive effect on seed-oil content this may be due to Zn mobilized by *B. japonicum* and Zn supplied by soil and foliar application of NPs, which is effect on oil quantity as a result of activation of NADPH dependent dehydrogenase included in fat synthesis by application of zinc. These findings were also documented by those founded by **Raghuwanshi *et al.* (2017)**.Additionally, the obtained results are in agreement with **Yacoub *et al*. (2020**) who found a positive effect of foliar applicetion of neutral rock P Nps+ $ZnSO_4$ or ZnO NPs recorded the maximum seed weight and straw yield, content of protein and protein (%) as well as content of oil and oil (%) of soybean**. Vaghar *et al*. (2021)** found that under drought conditions the lowest oil % at seed filling stage was (17.47%), while the highest oil % (23.73%) was recorded by foliar co-application of Zn and Mn nanoparticles in soybean plants.

The contribution of *B. japonicum*in in N-fixation, which is responsible for amino acid formation and many other important biological compounds that have essential role in photosynthetic process and subsequantly increase seed weight. This is in harmony with **Fituma *et al.* (2018) who** founded that the treated soybean plants with *Bradyrhizobium* recorded the highest 100-seeds weight (22 g), whereas the un-inoculated treatment (control) recorded the lowest 100-seeds weight (16.9 g).

## 5. Conclusions

Co-application of N & P& Zn &Fe NPs and *B. japonicum* showed positive effect on the growth and productivity of soybean in sand soil. The soil applications of *B. japonicum* enhanced minerals solubility in rhizosphere besides their production for exopolysaccharides that have a vital role in improve texture and properties of sandy soil. Additionally, both soil and foliar application of NPs and *B. japonicum* increased nutrient uptake and improved morphological and physiological traits of plant. Furthermore, the synergistic performance of NPs and rhizobium sp. increase chlorophyll content, carbohydrates percent and leaf area of soybean plant. The plant growth promoting traits of *B. japonicum* and NPs application provide highestquality and quantity of soybean seeds. From the results of these study, it was suggested that the soil addition and

foliar spray of N &P &Zn &Fe nanoparticles and *B. japonicum* could be recommended to improve soybean plants cope growth in sandy soil.

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