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# Interaction Effects of Foliar (ZnO-NP) Application with Both Phosphorus, Biofertilizer on Growth Parameters, Chemical Composition of Chickpea Plants (*Cicer arietinum* L.)

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**Abstract:** A field experiment was conducted during the successive seasons (2019 - 2020) in private farm Nubaria, Behaira Governorate, Egypt to study some interactions effect of foliar application with ZnO-NP, phosphorus and biofertilizer on growth parameters, chemical composition of chickpea plants (*Cicer arietinum* L). Results showed that utilization of phosphorous fertilizer with biofertilizers (rhizobium and/or phosphorous solubilizing bacterial inoculates (PSB) and foliar spray of zinc oxide Nano particals fertilizer stimulates the growth parameter and yield production of chickpea. Data revealed that application of nano fertilizers (ZnO-NP) gradually stimulate as well as improve the yield and its components. Interaction effect between combined inoculation for both rhizobium and phosphate-solubilizing bacteria gradually increase the growth parameters and yield production as compared with the individual inoculation of both rhizobium and bacterial solubilizing phosphate. Results also showed that the low productivity of chickpea particularly, in control treatment is mainly due to imbalance application of nutrients. Rhizobium and phosphate solubilizing bacteria (PSB) had shown advantage in enhancing chickpea productivity, cost effective, ecofriendly and renewable sources of plant nutrients.

**Key words:** Chickpea plants · Chemical composition · Biofertilisation · Rhizobium and PSB · Nodule formation

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is globally the third most important food legume after common bean and soybean [1, 2]. With increasing the population the developing countries cannot afford more expensive animal protein in particularly, various pulses play a crucial role to satisfy the growing human food demands and are also used as source for animal feed [3, 4]. Chickpeas may be a major protein source of essential nutrients like zinc, magnesium, niacin, vitamin C and β-carotene and amino acids [5, 6]. Chickpeas also, containing high fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes [7-10]. Their leaves contain malic acid, that is useful for stomach ailments and blood purification as well. Several researchers [11-16], reported that chickpea plays a significant role for sustainability of agricultural lands through a biological nitrogen fixation, as a rotational crop allowing the diversification of agricultural production systems and weed control.

Phosphorus fertilizers are also of the most important inputs particularly, in leguminous crops production. Application of phosphatic fertilization particularly, in adequate amounts is closely related to plant growth as well as developing and improving the quality of seeds. They're also enhancing and regulating photosynthesis and some physico-biochemical processes, as well as maintaining root enlargement and increasing nitrogen

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fixation and resistance to plant disease. [17-23] stated that low soil phosphorus and its poor utilization efficiency might be a major constraint limiting the productivity of most crop legumes. Biofertilizers seem to be a good option for sustainability of agricultural lands particularly, on a commercial and profitable scale. [24, 25] reported that biofertilizers are eco-friendly, easily available and cost effective. There are different types of microbial inoculants such as Rhizobium inoculants, Azotobacter, blue- green algae inoculants, azolla and phosphate solubilizing bacterial (PSB). [26] stated that phosphorus-solubilizing bacteria (PSB) plays a key role in the consistent capacity for increasing the availability of phosphates by mineralizing organic phosphorus compounds and act as bio control agents of phyto pathogenic fungi and produce phytohormones in the rhizosphere, which promote plant growth. [27] indicated that plant height, root length, phosphorus content & uptake, nodule number & weight gradually enhanced by inoculation with phosphate solubilizing bacteria. [28] observed that Rhizobium co-inoculation with Pseudomonas or Bacillus strains for bean plants, improved dry weight of shoot, nitrogen and phosphorus contents as compared with inoculation with Rhizobium alone, whereby, pseudomonas promoted bean growth and P uptake was more efficiently than Bacillus. Furthermore, [29] found that, the combination of Rhizobium inoculation and phosphate-solubilizing bacteria gradually increased nodulation, growth and yield parameters in chickpea. [30] stated that micronutrients are needed in small quantities for optimization the plant growth and play a major role in human health. Micronutrients such as zinc, iron, manganese and copper may provide a great role such as influencing the uptake and use potency of N; for increasing biomass production; and promoting tolerance to abiotic (drought and salinity stress) and organic phenomena (pests and diseases) and plant health particularly, underneath legume production conditions. [31-36]. Micronutrient deficiency may depress plant growth or perhaps complete inhibition on the yield of chickpeas [37]. Several researchers [38-46] reported that nano fertilizers are more soluble and more reactive than traditional fertilizers. Nano fertilizers may help the capability for improving the overall growth of the plants; reduce pollution and improvement soil fertility and making favorable environment for microorganism. Zinc oxide nano particles (ZnO-NP) is one of common additives and have various applications in agriculture. [47-54] found that application of Nano-fertilizers of zinc oxide (ZnO-NP) could increase growth promoter and yield of seeds of chickpea plant. The main objective of this study is increasing the yield and quality of chickpea through applying different combination of P, bio fertilizers and zinc nano particles.

## MATERIALS AND METHODS

A field experiment was conducted during the successive seasons (2019-2020) in private farm in Nubaria, Behaira Governorate, Egypt to study some interaction effects of foliar) application with zinc oxid nano praticals (ZnO-NP), phosphorus and biofertilizer on growth parameters, chemical composition of chickpea plants (*Cicer arietinum* L). Soil sample from the experimental field was air dried and passed through a 2mm sieve and stored for laboratory analysis. The characterization of the investigated soil were sand 89.3% silt 9.69% and clay 1.01 % with texture classes (sandy soil), pH 8.25, E.C. 0.15 $dSm^{-1}$, organic matter 0.35% , $CaCO_3$ 1.73% , available N (38ppm), available $P_2O_5$ (13ppm) and available potassium $K_20$ (5ppm). The physical and chemical properties of soil were determined according to [55, 56]. The experiment was laid out in a three-replicated in randomized complete block design (RCBD). Three seeds of chickpea (*Cicer arietinum* L.) cv. Giza were obtained from the department of Vegetable Crops, Agricultural Research Center, Ministry of Agriculture; Egypt and planted on 12$^{th}$ of November, at rate of 50 kg $fed^{-1}$. The plot size was 3 m x 3.5 m (10.5 $m^2$), with spacing of 1.2 m left between the experimental plots and 2.5 m was left between the blocks to prevent interactions among the treatments. Seeds were sown in rows by maintaining 30 cm and 10 cm between the rows and plants, respectively. Two seeds were sown per hill. Field was irrigated after planting to ensure uniform germination. Plants were thinned after 30 days from germination to one seedling per hill. All necessary agronomic practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. Chickpea plants fertilized with 20 kg $fed^{-1}$, ammonium sulphate (20.5%N) and 10 kg $fed.^{-1}$ potassium sulphate (48%$K_2O$) as a control treatment without phosphorous addition. Phosphorus in forms of triple superphosphate ($P_2O_5$) at a rate, 60 kg $fed^{-1}$, were added to the rest of experiments. Rhizobium inoculant at 30 g $kg^{-1}$ seed was applied as seed treatment, whereas, phosphorene (*Bacillus megaterium*) was utilized as the source of the P solubilizing bacteria (PSB) were applied in soil at 1.5 kg fed$^{-1}$. Foliar spray of ZnO as Nano fertilizers at a rate of 100 and 200 mg L$^{-1}$. Three sprays at 3 weeks intervals were used; first, one was applied after 45 days of cultivation.

The experiment includes ten treatments as follows:

- Control 20 kg N fed.$^{-1}$ and 20 $K_2O$ kg fed.$^{-1}$ +(water foliar spray )
- Phosphorus(P) + Rhizobium(R)
- Phosphorus(P) + Rhizobium (R) +100 mg/L of ZnO-NP
- Phosphorus(P) + Rhizobium(R) +200 mg/L of ZnO-NP
- Phosphorus(P) + P solubilizing bacteria) PSB
- Phosphorus(P) + P solubilizing bacteria) PSB (+100 mg/L of ZnO-NP
- Phosphorus(P) + P solubilizing bacteria) PSB (+20 0mg/L of ZnO-NP
- Phosphorus(P) + P solubilizing bacteria) PSB) + Rhizobium
- Phosphorus (P)+ Rhizobium + P solubilizing bacteria) PSB(+100 mg/L of ZnO-NP
- Phosphorus (P)+ Rhizobium + P solubilizing bacteria) PSB(+200 mg/L of ZnO-NP

**Measured Parameters:** At harvest, ten guarded plants were randomly taken from the central ridges in each plot to determine the following traits; Plant height, number of pods. Plant$^{-1}$, Leaf area. Plant$^{-1}$ (cm)$^2$, number of leaves, root length (cm), root, leaves and pods dry weight. Plant$^{-1}$ (g), total dry weight plant$^{-1}$ (g), number of seeds /pods, number of seeds. Plant$^{-1}$, weight of 100 seed of (g), weight of seeds. Plant$^{-1}$ (g), grain yield kg plot$^1$, seeds and straw yield Kg. fed$^{-1}$, biological yield Kg. fed$^{-1}$, cropindex and harvest index.

**Chemical Constituents:** Prior to digestion the samples were dried to, remove possible moisture gained before the analysis.

- Total N, P and K, in roots and leaves pods and seeds as well as some micronutrients (Zn), were determined using atomic absorption spectrophotometer, according to the method described by [57].
- Crude fiber, starch, Total sugar (%) and crude protein contents were determined according to the methods of [58].
- Nodule assessment was carried out as described by [59].

**Statistical Analysis:** Means of data recorded in the two successive seasons were subjected to the analysis of variance according to [60]. The least significant differences (LSD) at P = 0.05 level was used to verify the differences between among of the treatments.

## RESULTS AND DISCUSSIONS

Results of chickpea plants presented in Table (1 & 2) showed that the utilization of phosphorous fertilizers with biofertilizers (rhizobium and/or phosphorous solubilizing bacterial inoculates (PSB) and foliar spray of zinc oxide Nano fertilizers increased the growth parameters and yield production of chickpea plants comparing to the control treatments. Several researchers [61-64] reported that plant growth promoting rhizobacteria (PGPR) is a group of bacteria that can be found in the rhizosphere. The term plant growth promoting bacteria" refers to bacteria that colonize the roots of plants (rhizosphere) that enhance plant growth. Rhizosphere is the soil environment where the plant root is available and is a zone of maximum microbial activity resulting in a confined nutrient pool in which essential macro- and micronutrients are extracted.

Biofertilizers promotes the growth parameters through increasing the nutrients availability and supply to crop. Nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers and biomass accumulation. The impact of the microbial population particularly, in the rhizosphere is relatively different from that of its surroundings due to the presence of root exudates that function as a source of nutrients for microbial growth. It has been observed that narrow rhizosphere zone is rich in nutrients for microbes as compared to the bulk soil; this is shown by the quantity of bacteria that are present surrounding the roots of the plants, generally 10 to 100 times higher than in the bulk soil.

In addition, application of nano fertilizer (ZnO-NP) gradually stimulates and improve yield components and production. These improving were due to enhancing photosynthetic and other metabolic activity which leads to an increase in various plant metabolites responsible for cell division and elongation [65-68]. They stated that the enhancement of plant growth by the application of these microbial populations is well known and proven. The term "plant growth promoting rhizobacteria (PGPR)" for these beneficial microbes was introduced by paving the way for

Table 1: Effect of phosphorus fertilizer, biofertilizer and foliar spray of zinc oxide ZnO-NP on growth parameters of chickpea plants

| Treatments | Plant height (cm) | No. of pods plant$^{-1}$ | Leaf area plant$^{-1}$ (cm)$^2$ | No. of leaves | Root length (cm) | Root dry weight plant$^{-1}$ (g) | Leaves dry weight plant$^{-1}$ (g) | Pods dry weight plant$^{-1}$ (g) | Total dry weight plant$^{-1}$ (g) |
|---|---|---|---|---|---|---|---|---|---|
| Control(RDF ) | 45.30 | 13.48 | 440.2 | 29.16 | 14.15 | 0.88 | 2.94 | 5.37 | 8.69 |
| (P + R) + 0 mg/LZnO-NP | 50.61 | 16.22 | 580.62 | 35.22 | 15.30 | 1.46 | 3.88 | 5.61 | 10.92 |
| (P+R) +100mg/LZnO-NP | 54.11 | 21.67 | 671.35 | 38.64 | 16.30 | 1.50 | 4.69 | 6.64 | 12.83 |
| (P+R) +200mg/LZnO-NP | 60.30 | 19.11 | 680.81 | 40.11 | 17.00 | 1.48 | 5.15 | 7.53 | 14.11 |
| (P+ PSB) +0 ZnO-NP mg/L | 54.28 | 19.81 | 632.29 | 38.26 | 15.83 | 1.44 | 4.31 | 6.44 | 12.19 |
| (P+ PSB) +100 mg/LZnO-NP | 59.43 | 24.34 | 739.93 | 42.49 | 17.68 | 1.77 | 5.00 | 7.39 | 15.33 |
| (P+ PSB) +200mg/LZnO-NP | 64.60 | 22.72 | 744.84 | 45.05 | 17.30 | 1.56 | 5.25 | 7.71 | 16.81 |
| (P+ R+PSB) +0mg/LZnO-NP | 59.04 | 21.30 | 688.84 | 41.25 | 16.75 | 1.60 | 4.80 | 7.77 | 14.21 |
| (P+ R+ PSB) +100 mg/LZnO-NP | 66.85 | 25.08 | 788.60 | 45.08 | 18.38 | 1.85 | 5.85 | 8.12 | 16.09 |
| (P+ R+ PSB) +200 mg/LZnO-NP | 71.20 | 23.00 | 843.14 | 47.70 | 19.94 | 2.59 | 6.33 | 9.42 | 18.82 |
| LSD 5% | 4.09 | 2.12 | 47.44 | 3.27 | 1.28 | 0.27 | 0.66 | 0.67 | 1.14 |

P: phosphorus R: RhizobiumPSB: P solubilizing bacteriaControl:((RDF) without (P) added

Table 2: Effect of phosphorus, biofertilizer and foliar spray of zinc oxide ZnO-NP on yield of chickpea plants

| Treatments | No. of seeds /pod | No. of seeds plant$^{-1}$ | 100 seed weight (g) | Weight of seeds plant$^{-1}$ g | Seeds yield kg plot$^1$ | Seeds yield Kg fed$^{-1}$ | Straw yield Kg fed$^{-1}$ | Biological yield Kg fed$^{-1}$ | CI | HI |
|---|---|---|---|---|---|---|---|---|---|---|
| Control (RDF ) | 1.11 | 22.44 | 14.49 | 11.43 | 1.05 | 426.40 | 941.67 | 1367.96 | 0.45 | 31.17 |
| (P + R) + 0 mg/L ZnO-NP | 1.35 | 29.88 | 18.83 | 15.71 | 1.47 | 488.21 | 968.00 | 1456.34 | 0.50 | 33.52 |
| (P+R) +100 mg/L ZnO-NP | 1.44 | 36.50 | 20.04 | 16.90 | 1.60 | 593.90 | 1050.67 | 1644.44 | 0.57 | 36.15 |
| (P+R) +200 mg/L ZnO-NP | 1.61 | 33.75 | 21.32 | 16.00 | 1.53 | 553.70 | 1033.33 | 1587.03 | 0.53 | 34.89 |
| (P+ PSB) +0 ZnO-NP mg/L | 1.56 | 35.70 | 19.65 | 15.10 | 1.59 | 531.60 | 972.80 | 1504.40 | 0.55 | 35.29 |
| (P+ PSB) +100 mg/L ZnO-NP | 1.87 | 36.94 | 22.49 | 16.93 | 1.81 | 710.50 | 1188.66 | 1899.10 | 0.60 | 37.41 |
| (P+ PSB) +200 mg/L ZnO-NP | 1.90 | 37.97 | 23.81 | 17.11 | 1.74 | 681.30 | 1201.00 | 1882.13 | 0.57 | 36.20 |
| (P+ R+PSB) +0 mg/L ZnO-NP | 1.68 | 37.56 | 22.19 | 16.40 | 1.62 | 655.00 | 1221.00 | 1875.97 | 0.54 | 34.91 |
| (P+ R+ PSB) +100 mg/L ZnO-NP | 2.01 | 43.41 | 24.80 | 19.33 | 1.98 | 807.21 | 1289.00 | 2095.8 | 0.63 | 38.49 |
| (P+ R+ PSB) +200 mg/L ZnO-NP | 2.28 | 39.63 | 24.87 | 17.40 | 1.85 | 780.40 | 1336.67 | 2117.01 | 0.59 | 36.87 |
| LSD 5% | 0.20 | 3.80 | 2.28 | 1.59 | 0.30 | 47.20 | 44.46 | 63.71 | 0.04 | |

CI: crop index HI: harvest index

greater discoveries on PGPR. PGPR are not only molecules associated with the root to exert beneficial effects on plant development but also have positive effects on controlling phytopathogenic microorganisms Therefore, PGPR serve as one of the active ingredients in biofertilizer formulation.

Results also indicated that application of phosphorus fertilizer + solubilizing bacterial inoculates (PSB) gave the highest value of growth parameters and yield as compared to phosphorus fertilizer + Rhizobium [69, 70, 71]. The increase of growth parameters and yield may be due to the phosphorous-associated solubilizing bacterial inoculates (PSB) which produce adequate amounts of IAA and cytokinins and some volatile compound. Also, increase the synergistic activity of microbes for biological nitrogen fixation, which increases the surface area of root per unit, root length and enhances the root hair branching with an eventual increase in nutrient acquisition from the soil. [72] concluded that phosphate solubilizing bacteria (PSB) increase the solubilization of insoluble P compounds such as RPs through the production of organic acids and phosphatases. Insoluble phosphate compounds can be solubilized by organic acids and phosphatase enzymes produced by microorganisms. They also stated that, symbiotic bacteria mostly reside in the intercellular spaces of the host plant, but there are certain bacteria that are able to form mutualistic interactions with their hosts and penetrate plant cells. In addition to that, a few are capable of integrating their physiology with the plant, causing the formation of specialized structures. Rhizobia, the famous mutualistic symbiotic bacteria, could establish symbioticassociations with leguminous crop plants, fixing atmospheric nitrogen for the plant in specific root structures known as nodules.

Data presented in Table (3 & 4) indicated that the interaction effect of combined inoculation for both rhizobium and phosphate solubilizing bacteria gradually increase the growth parameters and yield production as compared with the individual inoculation of both rhizobium and bacterial solubilizing phosphate. Increment in plant height may be due to increasing nitrogen and phosphorus uptake by the plants, Results also indicated that the poor productivity of chickpea particularly, in control treatment is mainly due to imbalance application of nutrients. Under such situations, the use of rhizobium and phosphate solubilizing bacteria (PSB) had shown to enhance chickpea productivity. Rhizobium and PSB assume a great importance because of their vital role in $N_2$-fixation and P-solubilization. The rhizosphere of crops and soils has been reported to help in increasing phosphorus availability in the soil. These results are in agreement with those reported by [29, 73, 74, 75].

Table 3: Effect of phosphorus, biofertilizer and foliar spray of zinc oxide ZnO-NP on N, P and K, content (%) on roots, leaves, pods and seeds of chickpea plants

| Treatments | Roots | | | Leaves | | | Pods | | | Seeds | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | N | P | K | N | P | K | N | P | K |
| | | | | | | % | | | | | | |
| Control (RDF ) | 0.56 | 0.14 | 0.77 | 0.88 | 0.28 | 0.90 | 1.39 | 0.35 | 1.02 | 1.84 | 0.40 | 1.18 |
| (P + R) + 0 mg/L ZnO-NP | 0.62 | 0.18 | 0.84 | 0.92 | 0.31 | 1.18 | 1.56 | 0.40 | 1.21 | 2.50 | 0.48 | 1.36 |
| (P+R) +100 mg/L ZnO-NP | 0.66 | 0.22 | 0.96 | 1.13 | 0.35 | 1.18 | 1.77 | 0.46 | 1.30 | 2.66 | 0.55 | 1.45 |
| (P+R) +200 mg/L ZnO-NP | 0.71 | 0.19 | 0.90 | 1.08 | 0.33 | 1.35 | 1.63 | 0.44 | 1.44 | 2.82 | 0.51 | 1.61 |
| (P+ PSB) +0 ZnO-NP mg/L | 0.78 | 0.22 | 1.20 | 1.12 | 0.33 | 1.27 | 1.90 | 0.46 | 1.32 | 2.89 | 0.53 | 1.57 |
| (P+ PSB) +100 mg/L ZnO-NP | 0.82 | 0.27 | 1.15 | 1.21 | 0.38 | 1.32 | 2.58 | 0.50 | 1.44 | 3.09 | 0.68 | 1.76 |
| (P+ PSB) +200 mg/L ZnO-NP | 0.82 | 0.25 | 1.22 | 1.18 | 0.35 | 1.52 | 2.50 | 0.55 | 1.60 | 3.35 | 0.60 | 1.77 |
| (P+ R+PSB) +0 mg/L ZnO-NP | 0.83 | 0.26 | 1.10 | 1.22 | 0.35 | 1.56 | 2.00 | 0.51 | 1.64 | 3.17 | 0.65 | 1.64 |
| (P+ R+ PSB) +100 mg/L ZnO-NP | 0.91 | 0.30 | 1.40 | 1.50 | 0.40 | 1.64 | 2.71 | 0.65 | 1.77 | 3.49 | 0.78 | 2.28 |
| (P+ R+ PSB) +200 mg/L ZnO-NP | 0.85 | 0.30 | 1.33 | 1.40 | 0.37 | 1.81 | 2.52 | 0.57 | 2.00 | 3.60 | 0.68 | 2.40 |
| LSD 5% | 0.12 | 0.03 | 0.13 | 0.12 | 0.06 | 0.10 | 0.27 | 0.06 | 0.19 | 0.24 | 0.07 | 0.34 |

Table 4: Effect of phosphorus, biofertilizer and foliar spray of zinc oxide ZnO-NP on N, P and K uptake (mg/plant) on roots, leaves, pods and seeds of chickpea plants

| Treatments | Roots | | | Leaves | | | Pods | | | Seeds | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | P | K | N | P | K | N | P | K | N | P | K |
| | | | | | | (mg/plant) | | | | | | |
| Control (RDF ) | 4.96 | 1.20 | 6.78 | 25.97 | 8.23 | 26.56 | 74.82 | 18.80 | 51.44 | 209.93 | 45.72 | 135.25 |
| (P + R) + 0 mg/L ZnO-NP | 9.00 | 2.67 | 12.26 | 35.70 | 12.15 | 45.66 | 87.70 | 22.25 | 68.07 | 405.11 | 74.89 | 213.13 |
| (P+R) +100 mg/L ZnO-NP | 9.95 | 3.30 | 14.35 | 53.00 | 16.41 | 55.34 | 117.31 | 30.54 | 86.50 | 450.10 | 92.95 | 245.61 |
| (P+R) +200 mg/L ZnO-NP | 10.46 | 2.86 | 13.37 | 55.45 | 17.00 | 69.53 | 122.49 | 32.88 | 109.84 | 450.67 | 81.60 | 258.13 |
| (P+ PSB) +0 ZnO-NP mg/L | 11.23 | 3.17 | 17.33 | 48.41 | 14.08 | 54.74 | 122.14 | 29.84 | 85.22 | 436.89 | 80.03 | 236.57 |
| (P+ PSB) +100 mg/L ZnO-NP | 14.58 | 4.72 | 20.35 | 60.33 | 18.83 | 66.17 | 190.66 | 36.95 | 106.41 | 524.70 | 114.56 | 297.97 |
| (P+ PSB) +200 mg/L ZnO-NP | 12.79 | 3.90 | 19.03 | 61.95 | 18.55 | 79.63 | 192.75 | 42.15 | 123.62 | 573.18 | 103.23 | 302.85 |
| (P+ R+PSB) +0 mg/L ZnO-NP | 13.23 | 4.11 | 17.60 | 58.56 | 16.96 | 75.04 | 155.66 | 39.55 | 127.83 | 520.43 | 107.06 | 268.96 |
| (P+ R+ PSB) +100 mg/L ZnO-NP | 16.84 | 5.61 | 25.90 | 87.75 | 23.40 | 96.14 | 219.78 | 52.51 | 143.99 | 674.62 | 150.13 | 440.72 |
| (P+ R+ PSB) +200 mg/L ZnO-NP | 22.02 | 7.86 | 34.36 | 88.83 | 23.21 | 114.4 | 237.07 | 53.69 | 188.47 | 626.61 | 117.74 | 417.60 |
| LSD 5% | 2.16 | 0.45 | 2.08 | 6.08 | 3.30 | 5.74 | 17.21 | 6.45 | 15.01 | 41.16 | 11.4 | 34.30 |

It is clearly demonstrated from data in Table (2 and 3) that application of zinc oxide nano fertilizers at rates of 100 and 200 mg/L in combined with phosphorus + Rhizobium + P solubilizing bacteria) PSB), significantly increased the growth parameters and yield production as compared with phosphorus + P solubilizing bacteria) PSB) + Rhizobium without ZnO-NP. [76, 77, 78], stated that application of nano-fertilization stimulated the solubility and dispersion of insoluble nutrients in soil, which may reduce the nutrient immobilization and furthermore increasing their bio-availability.

Concerning the effect of different rates of ZnO-NP at rates of 100 and 200 mg/L with phosphorus + Rhizobium + P solubilizing bacteria) PSB) in chickpea. Data revealed that application of 100 mg/L ZnO-NP fertilizer, tended to increase seed and straw yield per plot.

However, application of 200 mg/L ZnO-NP fertilizer stimulate the number of seeds /pods, number of seeds plant$^{-1}$ and weight of 100 seeds. On the other hand, most the growth parameter gradually reduced. Such phenomenon may be due to the antagonistic reaction between zinc and phosphorus.

**Chemical Composition:** Results in Table (3 & 4) showed that application bio-fertilization alone or combining with zinc nano gradually stimulate the concentration and uptakes of N, P, K, contents and uptake in roots, leaves, pods and grain of chickpea plants over the control. Effeteness of biofertilizers related to the effect of different strains groups of microorganisms such as nitrogen fixer, nutrient-mobilizing microorganisms that stimulating and increasing the availability of minerals and consequently, increasing their uptake. [79-82] reported that the growth, development and yield of chickpea (*Cicer arietinum* L.) are strongly influenced by abiotic factors such as salinity and drought in the arid conditions. The use of efficient plant growth promoting bacteria in chickpea production is the best solution to overcome those stresses that play important role in the plant assimilation rate, which in turn increased N, P and K. Data in Tables 3 and 4 showed that application of phosphorus + Rhizobium + P solubilizing bacteria) PSB) with 100ml/ L of ZnO-NP foliar application gave the highest value of N% in roots leaves and pods as well as nitrogen content as compared with the other applied treatments, similar results observed by [83]. Whereas phosphorus + Rhizobium + P solubilizing bacteria) PSB) with 200ml/ L of ZnO-NP foliar application gave the highest value of N% in seeds. The beneficial effects of biofertilizers as well as application of nanoparticles in the field of agriculture can increase crop production. Nanoparticles can also decrease mineral loss and can significantly increase the nutrient use efficiency (*NUE*) and lessen the fertilizer use.

Similar trend was observed in the case of K%, such increments in nutrients concentration and uptake in grain and straw could be due to well developing root architecture, that helping for increasing nitrogen fixation as well as other nutrient elements to chickpea plants. In addition, as results of stimulating effect of ZnO-NP application on microbial ammonification or nitrification rates via influencing urease, dehydrogenase and nitrification enzymes activities [84, 85]. Nanotechnology is a promising field of research that has the potential to offer sustainable remedies to pressing challenges confronted to modern intensive agriculture. Nanotechnology employs nanomaterials that typically have the size of 1–100 nm and this small size imparts unique characteristics and benefits to nanomaterials. In addition to numerous other benefits, large surface area offers opportunity for better and effective interaction of nanoparticles to target sites. Nano fertilizers hold potential to fulfill plant nutrition requirements along with imparting sustainability to crop production systems and that too without compromising the crops yield.

It was observed that application of phosphorus fertilizer combined with solubilizing bacterial inoculates (PSB), gave slightly increase in phosphorus content in leaves (0.33%) and seeds (0.53%) comparing with phosphorus fertilization in combined with rhizobium. Data also showed that average increment was (0.31%) in leaves and (0.48%) seeds whereas control treatment gave slightly increase by about (0.28%), (0.40%) in leaves and seeds, respectively. The significant increasing trend of phosphorus content and uptake by seeds may due to increased concentration of phosphorus in soil solution with increasing phosphorus application and increase in biological activity by P-solubilization [86, 87, 88]. The interactive effect between phosphorus fertilizers in combining with solubilizing bacterial inoculates (PSB) and Rhizobium as biofertilizer and foliar spry with ZnO nano-fertilizer at a rate of 100 mg/L was found to have significant effect on phosphorus content in leaves and seeds as compared to the other treatment (200 mg/L). Results also indicated that, antagonistic phenomenon as well as interaction in soil could play an important part for a reduction of phosphorus content.

Zinc content in both seeds and leaves of chickpea plant, gradually increased due to (Rhizobium + P solubilizing bacteria) and ZnO nano-fertilizer application, at a rate of 200 mg/L of ZnO-NP [89]. The uptake of zinc in seeds increased up to some level due to increased biomass of crop and less formation of zinc phosphate.

Table (5), illustrate the effect of phosphorus, biofertilizers and foliar spray of nano zinc oxide (ZnO-NP) on content of protein %, Total sugar (%), seeds crude fiber (%), starch% and Zn content and uptake in seeds of chickpea plants the content of protein %. Results showed that total sugar (%), seeds crude fiber (%) and starch % in seeds of chickpea plants were significantly increased with application treatments as compared to the control one-control treatment similar observation were noticed by [90].

[91] stated that the foliar application of inorganic nano-materials on cereal plants during their growth cycle enhances the rate of plant productivity by providing a micronutrient source. Application of ZnO-NP stimulated plant grains and had significantly higher oil and total nitrogen contents. However, such treatments have significantly lower crop water stress index (CWSI). This highlights that the slow-releasing nano-fertilizer improves plant physiological properties and various grain nutritional parameters and its application is therefore beneficial for progressive nanomaterial-based industries.

Data in Table (5), also showed that seed inoculation with rhizobium bacteria may stimulated the nitrogen content in seed of chickpea, therefore increasing in higher protein content as compared to the control treatment. A significant effect of base fertilizers on protein, crude fiber and starch content of chickpea grain wasobserved, similarly for starch content of seeds were gradually increased by the combination between biofertilizer and zinc nano compared to individual treatments. [92, 74] stated that Nano fertilizers have been provided a new efficient alternative to normal regular fertilizers. Nanoparticles can help in increasing reactive points of these nanoparticles, which increases the absorption of these fertilizers in plants. They reported that treatment of chickpea plant with either ZnO or nano ZnO gradually stimulate and improved the growth parameters, biochemical aspects and consequent yield.

Table 5: Effect of phosphorus, biofertilizer and foliar spray of Zinc oxide ZnO-NP on content of protein %, Total sugar (%), seeds crude fiber (%), starch% and Zn content and uptake in seeds of chickpea plants

| | Seeds | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | Zinc | | |
| | Protein | Total sugar | Crude fiber | Starch | | | |
| Treatments | (%) | | | | ppm | Uptake (μg/ plants) | No of nodules plant$^{-1}$ |
| Control (RDF) | 11.50 | 4.40 | 5.00 | 25.30 | 25.47 | 291.16 | 23.80 |
| (P + R) + 0 mg/L ZnO-NP | 15.60 | 4.90 | 5.20 | 28.00 | 30.11 | 472.97 | 25.30 |
| (P+R) +100 mg/L ZnO-NP | 16.60 | 5.30 | 5.70 | 28.90 | 33.46 | 565.53 | 27.84 |
| (P+R) +200 mg/L ZnO-NP | 17.60 | 5.50 | 5.70 | 30.30 | 36.04 | 576.59 | 31.81 |
| (P+ PSB) +0 ZnO-NP mg/L | 18.10 | 5.10 | 6.20 | 28.60 | 33.40 | 504.29 | 28.37 |
| (P+ PSB) +100 mg/L ZnO-NP | 19.20 | 5.89 | 6.80 | 30.10 | 42.18 | 714.11 | 30.10 |
| (P+ PSB) +200 mg/L ZnO-NP | 20.90 | 6.00 | 6.60 | 31.60 | 48.31 | 826.64 | 38.00 |
| (P+ R+PSB) +0 mg/L ZnO-NP | 19.80 | 5.55 | 7.00 | 30.40 | 35.29 | 578.81 | 35.27 |
| (P+ R+ PSB) +100 mg/L ZnO-NP | 21.80 | 6.20 | 7.60 | 34.60 | 45.26 | 874.88 | 43.60 |
| (P+ R+ PSB) +200 mg/L ZnO-NP | 22.50 | 6.60 | 8.10 | 36.80 | 54.26 | 944.12 | 49.80 |
| LSD 5% | 1.30 | 0.93 | 1.12 | 2.83 | 2.97 | 52.70 | 2.03 |

Data also, in Table 5 showed that phosphorus fertilizer in the presence of biofertilizer and foliar spry ZnO nano-fertilizer at a rate of 200 mg/L had more pronounced effect for protein content than phosphorus fertilizer with biofertilizer and foliar spray ZnO nano-fertilizer at a rate of 100 mg /L.similarly were noticed by [93].

Table (5) showed that inoculated seed of chickpea by PSB + Rhizobium with ZnO nano fertilizer, gave the highest total sugar of seeds value (6.6 %), however, the lowest value was noticed in the treatment of rhizobium + ZnONano fertilizer (5.5%). Data in Table (5) indicated that higher nodule number plant$^{-1}$, observed in the seeds inoculated with rhizobium and PSB . Application of zinc nano at a rate of 100 mg /L. and 200 mg /L. gave the highest number of nodules. This might be due to the fact that application of phosphorus results profuse growth of roots, which ultimately resulted formation of number of nodules of enormous size. PSB + Rhizobium inoculation significantly increased the quantity of nodules plant$^{-1}$ mainly because the nitrogenase enzyme present within the bacteria is introduced through infection causes nodule formation. An application of PSB facilitates the basis development vis-à-vis nodule formation and proper development of nodules by increasing the supply of phosphorus through the mobilizing the unavailable phosphorus present within the soil .Similar result has been recorded by [29, 94, 95, 96].

## CONCLUSION

Soils contain natural reserves of plant nutrients but these reserves are largely in different forms, which are used for plant growth. In addition, such nutrients are not available to plants, because of the extra use of the chemical fertilizers. Chemicals make the nutrients, which remain in soil, in active and so are not used by plants make the soil polluted. Using of chemical fertilizer since long time results in the soil being full of chemicals thus, damaging the production and full of harmful chemicals to the human body. As an option to all this use of biofertilizers can help us get back our soil health by natural way ultimately the health of organisms. Biofertilizers help to increase quality of the soil by providing nutrients and natural environment in the rhizosphere. The microorganisms present in biofertilizers are important because they produce nitrogen, potassium, phosphorus and other nutrients required for benefit of the plants. Most biofertilizers also secrete hormones like auxins, cytokinins, biotins and vitamins, which are essential for plant growth. Biofertilizers are inexpensive and safe inputs that provide a wide scope for research in the areas of organic farming and development of stress-free environment. The efficiency of nutrient use in a crop production can be enhanced by the effective use of nano-fertilizers. Nano fertilizers improve crop growth and yield to optimum doses and concentrations, but they also have an inhibitory effect on crop plants if the concentration is more than optimal and the result reduces crop growth and yield.nano fertilizers have been provided a new efficient alternative to normal regular fertilizers. Nanoparticles can help in increasing reactive points of these nanoparticles, which increases the absorption of these fertilizers in plants. They reported that treatment of chickpea plant with either ZnO or nano ZnO gradually stimulate and improved the growth parameters, biochemical aspects and consequent yield.

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