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INFLUENCE OF IRON, ZINC AND BIMETALLIC ZN-FE NANOPARTICLES ON GROWTH AND BIOCHEMICAL CHARACTERISTICS IN CHICKPEA (*Cicer arietinum*) Cultivars

SUMMARY

Nano-fertilizers in agriculture are becoming prevalent because of the unique and outstanding properties of these materials. Bimetallic nanoparticles can simultaneously provide essential elements for plants. A field experiment was conducted on a semi-arid region at Maragheh, northwest of Iran to study effects of foliar application of Fe_3O_4 nanoparticles (Fe-NPs), ZnO nanoparticles (Zn-NPs), mixed monometallic Fe_3O_4 + ZnO nanoparticles, bimetallic Fe-Zn nanoparticles and distilled water (as control) on biochemical attributes of two chickpea cultivars (Gogso and local). The tallest plant was recorded for cv. Gogso by application and Zn-NPs and Fe_3O_4+ ZnO nanoparticles. Evaluation of seed yield component such as 100-seed weight, pod and seed number per plant showed that foliar spry of bimetallic Fe-Zn nanoparticles significantly increased the agro-economic performance especially in cv. Gogso. Assessment of leaf chlorophyll a, b and carotenoids content showed that although the additive effect of Fe-NPs was greater than the Zn-NPs, the highest pigments content was recorded for cv. Gogso by utilization of bimetallic Fe-Zn. Also, antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD) and guaiacol-peroxidase (GPX) significantly induced by application of micronutrients and highest activity was recorded for prayed by bimetallic Fe–Zn. This was confirmed by a significant reduction in hydrogen peroxide (H_2O_2) concentration. Our results showed that cv. Gogso was more responsive to composite micronutrient nanoparticles than local cultivar. This highlights that the bimetallic Fe-Zn nanoparticles improves plant physiological properties, seed yield, and its utilization is therefore especially beneficial for progressive nano-fertilizer industries.

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INTRODUCTION

Chickpea is an important pulse crop widely used for food and fodder throughout the world. It is one of the main sources of protein for majority of the population in developing countries among all pulse crops. The world's chickpea consumption is third after dry beans and peas (*Pisum sativum* L.) and has shown an increase of 14.2% in area and 27.3% in quantity of production since 2010 (FAOSTAT, 2019). It is a good source of carbohydrates and protein, and also its protein quality is considered to be better than other pulses (Merga and Haji, 2019). Also, it is adaptable to wide climatic variation, has low production cost, and promotes biological fixation of atmospheric nitrogen (Pegoraro *et al*., 2018). Hence it can be included in crop rotations of semi-arid areas.

Arid and semi-arid regions account for more than 30% of the total earth surface (Liu *et al*., 2018). The soils of the arid and semi-arid area are increasing agricultural importance, but their inherent fertility is low. Also, micronutrients availability is much affected by soil pH. So that in alkaline soils the availability of zinc, iron, manganese and copper is reduced and so, plants exhibit strong deficiency symptoms. On the other hand, population growth pressure and agricultural intensification in this region poses a serious threat to agricultural systems and may increase the degree of desertification and decrease soil quality (Mohammed *et al*., 2021). Food security in semi-arid regions is generally influenced by water and nutrient management.

Zinc (Zn) deficiency is mostly recognized from the countries where cereal-based food is dominant (Cakmak, 2008; Hacisalihoglu, 2020). Zn becomes the fourth most important nutrient after nitrogen (N), phosphorus (P), and potassium (K), and it is deficient in the 47% of the Iran soils (Boostani *et al*., 2019). Zn is structural part of many enzymes that are used in the metabolism of auxin and carbohydrates, in the synthesis of proteins and integrity of membrane (Tsonev and Cebola Lidon, 2012; Umair Hassan *et al*., 2020). Furthermore, it also has critical roles in pollen development, fertilization and chlorophyll synthesis (Karim *et al*., 2012; Hacisalihoglu, 2020). Iron (Fe) is an essential micronutrient for almost all living organisms because it plays important role in metabolic processes such as photosynthesis, DNA synthesis and respiration (Schmidt *et al*., 2020). Further, many metabolic pathways are activated by iron, and it is a central component of electron chains and a co-factor of many vital enzymes. Although Fe is the fourth most abundant element in the earth's crust, it is the third-most limiting nutrient for plant growth primarily due to the low solubility of the oxidized ferric form in aerobic environments (Zuo and Zhang, 2011). Limited Fe availability in soils is one of the main limiting factors of yield and quality of agricultural productions worldwide, particularly in alkaline and calcareous soils of semi-arid regions (Mohammed *et al*., 2021). This poor availability is closely linked to physical, chemical, and biological processes within the rhizosphere as a result of soil–microorganism–plant interactions. Iron shortage in plants might be prevented by the foliar application of Fe fertilizers where the soil conditions are unsuitable.

Recently, nano-technology has been widely employed for the production of fertilizers due to the high efficiency and the homogenous distribution of nano-form of the nutrients within the plants. Nanotechnology is the application of beneficial particles at the atomic or molecular level, usually at scales <100 nm. In recent years, research into nano-fertilizers has increased. Nano-fertilizers can be more soluble or more reactive than conventional fertilizers (Konappa *et al*., 2021; Mejías *et al*., 2021). It appears that new generation of fertilizers based on nano-technology has a potential to provide solutions to fundamental agricultural problems caused by conventional fertilizer management (Mastronardi *et al*., 2015).

Nano-fertilizers have been provided a new efficient alternative to normal regular fertilizers. Nano-particles can help in increasing reactive points of these nanoparticles, which increases the absorption of these fertilizers in plants (Sadak and Bakry 2020). According to the type of formulation, nano-fertilizers are classified into three categories: 1) nanoscale fertilizer, which corresponds to the conventional fertilizer reduced in size typically in the form of nanoparticles; 2) nanoscale additive fertilizer, is a traditional fertilizer containing a supplement nanomaterial; 3) nanoscale coating fertilizer, refers to nutrients encapsulated by nano-films or intercalated into nanoscale pores of a host material; and 4) Bimetallic nanoparticles (BNPs), which are formed by the combination of two different metals. Bimetallic nanoparticles have attracted huge attention as compared to monometallic nanoparticles in both technological and scientific view because BNPs shows better properties. (Mastronardi *et al*., 2015; Sharma *et al*., 2019).

However, the evaluation of the exact effects of foliar applying micronutrients nano-fertilizers individually, mix of different monometallic or in the form of bimetallic nanoparticles on chickpea crops in semi-arid region need to be better established. The present investigation was undertaken to improve the understanding about the foliar application of Fe and Zn nanoparticles on the growth and biochemical characteristics of spring chickpea in a Mediterranean semi-arid environment.

MATERIAL AND METHODS

Experimental site and plant material

A field experiment was carried out during 2019‒2020 growing season in semiarid highland region of Hashtroud (latitude: 37°28′N; longitude: 447°02′E; altitude: 1815 m) in northwest of Iran. Seeds of two chickpea cultivars (local and Gogso) were obtained from Dryland Agricultural Research Institute (DARI) and during the first-year seeds were propagated in isolated fields under full irrigated condition, according to Sabaghnia *et al*., (2015). The experiment was established on a silt loam soil (75% silt, 15% clay, 10% sand), with pH 7.63, low in organic carbon (0.64%), total nitrogen 0.08%, CaCo3 9.3%, electrical conductivity (EC)

1.62 ds m^{-1}, iron 2.89 ppm, manganese 1.02 ppm, zinc 0.44 ppm and potassium 363 ppm. The previous crop in the experimental field was wheat. The climate of Hashtroud is temperate and the winter months are much rainier than the warm months. According to Köppen-Geiger climate classification, the climate of this region is BS (cold semi-arid). The average annual temperature is 13.8°C in Hashtroud and its mean annual rainfall is 428 mm. Typically, during the spring-sown chickpea crop cycle, the amount of rainfall is usually very low. The precipitation was 79.8 mm during the cropping season. The experimental field was ploughed twice: in early autumn and two weeks before planting, subsequently soil was harrowed twice to bring the soil to fine tilth. After the second primary tillage operation, well-rotten farmyard manure was applied as per the treatment and thoroughly mixed into the topsoil. The recommended dose of fertilizer 60 kg ha^{-1} of nitrogen and 30 kg ha^{-1} of phosphorus was applied in the form of urea and triple superphosphate at the time of seed bed preparation. Cut worm (*Agrotis ipsilon*) was controlled by poison bait (Dipterex [trichlorfon; 200g] + sugar; 200g + wheat bran; 5kg). After opening of furrows, the seeds were hand planted on 11 April 2019.

Experimental design and plant growth measurement

The trial was laid out in randomized complete block design with three replications in split plot arrangement (plot size of 2 × 2 m^2) keeping foliar application of micronutrient fertilizers in main plots and sub-plots allocated to the cultivars (local and Gogso). Micronutrient fertilizers were including Fe_3O_4 nanoparticles (Fe-NPs), ZnO nanoparticles (Zn-NPs), Fe_3O_4+ ZnO nanoparticles, bimetallic Fe-Zn nanoparticles and foliar spray of distilled water as control. The rows were 35 cm apart with the plant-to-plant distance of 5 cm. A sprinkler system was applied for irrigation. Nano metal oxides were obtained from the Iranian Nanomaterials Pioneers Company. The synthesized nano particles were characterized morphologically by transmission electron microscope (Zeiss EM 10 C, Merck, Darmstadt, Germany). The plants were sprayed with the spraying liquids (2mM) until their leaves reached the point of maximum liquid retention, after which runoff occurred. Foliar treatments were applied at inflorescence emergence stage BBCH= 51 (first flower bud visible outside leaves) and repeated every four days for four times. Seed yield and yield component were measured from a 2.0 m^2 harvest area from the central four rows of each plot when the crop reached physiological maturity.

Antioxidant enzyme assay

For biochemical analysis leaf samples was collected at fruit development stage (BBCH= 71) and freshly harvested plant material immediately was freeze in liquid nitrogen and store it at -70°C for later use. For catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and guaiacol-peroxidase (GPX) extraction, leaf samples (0.5g) were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay as described previously by Janmohammadi (2012). CAT activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H2O2 and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. Guaiacol peroxidase (GPX) was determined by measuring the oxidation of guaiacol. The assay mixture contained 10 mmol/L potassium phosphate (pH 6.4), 8 mmol/L guaiacol, and 2.75 mmol/L H_2O_2. The increase in absorbance was recorded at 470 nm within 2 min (linear phase) after the addition of H_2O_2.

Hydrogen peroxide (H_2O_2) concentration was determined according to Loreto and Velikova (2001). Leaf samples of 0.5 g were homogenized in 3 mL of 1% (w/v) tri-chloroacetic acid. The homogenate was centrifuged at 10,000 rpm and 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI. H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm to a standard calibration curve. The concentration of H_2O_2 was calculated from a standard curve plotted in the range from 100 to 1000 μmol mL^{-1}. H_2O_2 concentration was expressed as μmol g^{-1} DW. Activities of SOD and APX was calculated according Cakmak and Marschner (1992).

Pigments content

Photosynthetic leaf pigments, chlorophylls a (chl a) and b (chl b) and total carotenoids x+c were quantified by the method of Lichtenthaler (1987) through a spectrophotometer at 661.6 nm, at 644.8nm and at 470 nm. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute 1988), the least significant difference (LSD) being used to compare means of traits ($p < 0.05$).

RESULTS AND DISCUSSION

The results indicated independent significant effects of nano-elements foliar applications and cultivars on plant height as well as significant effects were found by the cultivar × foliar sprays interactions (Table 1). Application of Fe and Zn both individually and in combined form increased plant height. However, combined application of micronutrient in the form of bimetallic structure increased plant height by about 18%. The tallest plant recorded for cv. Gogso by foliar application of bimetallic nanoparticles. In plant systems many metabolic pathways are activated by Fe and Zn, and it is a prosthetic group constituent of many enzymes. They also play some important roles in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants. All mentioned process individually can increase height by increasing the supply of photoassimilates or by cross talking with phytohormones and stimulants of plant growth. Phytohormone signaling plays crucial roles in regulating the response to variable mineral availability. High pH and precipitation lead to Zn deficiency in soil.

Evaluation of seed yield component showed that there is significant difference between the pod number of the cultivars. The pod number in cv. Gosgo was 19% higher than local cultivar. This indicates that the source-sink relationship in the new cultivar has been qualified to a considerable extent. However, grain yield and grain number of legumes are very sensitive to the assimilate availability during the post-flowering period. Therefore, it seems that the cv. Gosgo with higher plant height, more leaf area and larger canopy width has been able to produce more amounts of photoassimilates in the pre-flowering stages. The higher number of primary branches in Gosgo cultivar compared to the local cultivar also confirms this point (Table 1).

Table 1. Means comparison of morphological characteristics and pigments content of chickpea (Cicer arietinum L.) cultivars as affected by different nano-form micronutrients.

	PH	PB	PN	SP	HSW	Chl a	Chl b	CAR
C	29.00[bc]	3.50[b]	50.50[a]	1.00[a]	36.33[d]	90.00[e]	141.50[d]	467.33[cd]
Fe	31.00[ab]	4.33[a]	51.83[a]	1.50[a]	39.34[bc]	117.17[bc]	184.33[c]	522.67[c]
ZN	30.50[b]	3.83[b]	51.40[a]	1.50[a]	40.09[b]	104.19[d]	164.33[c]	509.67[c]
Fe+Zn	33.66[a]	4.50[a]	53.83[a]	1.50[a]	41.67[b]	129.17[b]	202.33[b]	683.67[ab]
Bimetal	34.17[a]	4.50[a]	54.33[a]	1.67[a]	44.17[a]	141.79[a]	317.67[a]	864.87[a]
Local	26.20[b]	3.33[b]	48.07[b]	1.4[a]	39.27[b]	120.80[a]	198.67[a]	645.73[a]
Gogso	37.13[a]	5.00[a]	56.80[a]	1.4[a]	42.20[a]	120.60[a]	205.27[a]	623.73[a]
Micronutrients (M)	**	Ns	Ns	Ns	**	**	**	**
Cultivar (C)	**	**	**	Ns	**	Ns	Ns	Ns
M×C	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns

PH: plant height (cm), PB: primary branch, PN: number of pods per plant, HSW: 100-seeds weight (g), Chl a: chlorophyll a content (mg g^{-1} FW), Chl b: chlorophyll b content (mg g^{-1} FW), CAR: carotenoids content (mg g^{-1} FW). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe_3O_4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe_3O_4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. In each ccolumns rows with different letters are statistically different at the 5% level. Ns: non-significant, *: Significant difference at P ≤ 5%, following two-way ANOVA, **: Significant difference at P ≤ 1%, following two-way ANOVA.

Assessment of 100-seed weight showed that this component affected by foliar treatment and heaviest seed was obtained by application of mixed Zn+Fe and bimetallic nanoparticles which improved seed weight by 14% and 21% over the control. Also seed weight affected by cultivar effects and in cv. Gosgo was higher than local cultivar. However, the interaction of cultivar × foliar sprays was non-significant (Table 1). Interaction effects of cultivar and foliar spray was significant for number of seed per plants. Although the use of micronutrients could improve number of the seeds in both cultivars, the rate of increase with the use of mixed Zn+Fe and bimetallic nanoparticles in Gosgo cultivar was more prominent than the local cultivar (Figure 1). So that foliar application of bimetallic nanoparticles in cv. Gosgo and local increased number of seed per plants by 34% and 22% over control. Better response cv. Gosgo to foliar application of micronutrient can be attributed to better canopy growth, taller stem, more leaf area and better micronutrient use efficiency. Growing micronutrient-efficient genotypes contributes to environmentally-benign agriculture by lowering the input of chemicals and energy. The greater rates of short-term micronutrient uptake and their transport is important factor in genotypic response. However, mechanisms conferring micronutrient efficiency are diverse, complex and still poorly understood (Khoshgoftarmanesh *et al*., 2010).

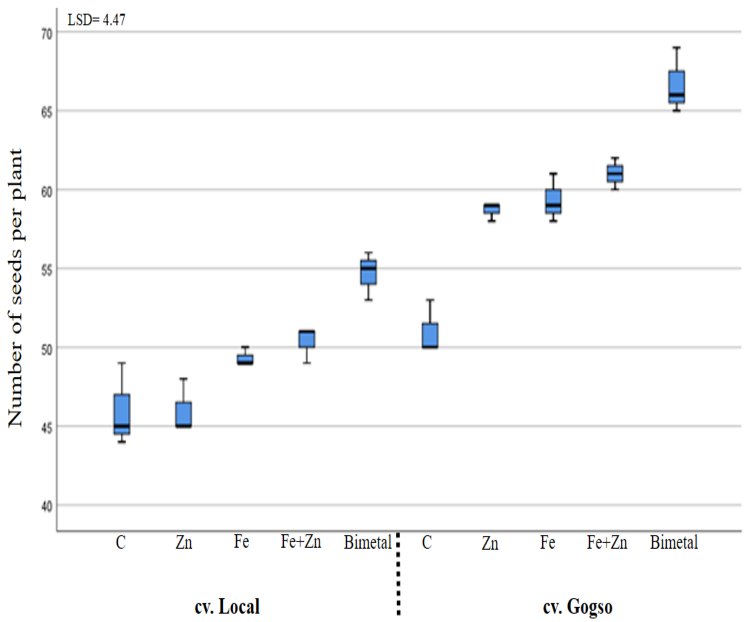

Figure 1. Effects of foliar application of different nano-form micronutrients on number of seeds per plant in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

A similar trend also was recorded for seed yield (Figure 2). In addition to the effectiveness of binary compounds, grain yield in cv. Gosgo was significantly increased by the use of Fe nanoparticles. Contrary to the findings of Niyigaba *et al*., (2019) we did not find any antagonist effects between applied micronutrient. Our finding showed that applying these micronutrients in combined form was better than separately utilization to increase seed yield. The result emphasized that high-yielding varieties need more micronutrients, which should be supplied through the combination of micronutrients fertilizers. Nano-pores and stomatal openings in plant leaves facilitate nanomaterial uptake and their penetration deep inside leaves leading to higher nutrient use efficiency. Nano-fertilizers have higher transport and delivery of nutrients through plasmodesmata, which are nanosized (50–60 nm) channels between cells. The higher NUE and significantly lesser nutrient losses of nano-fertilizers lead to higher productivity (6–17%) and nutritional quality of field crops (Iqbal, 2019).

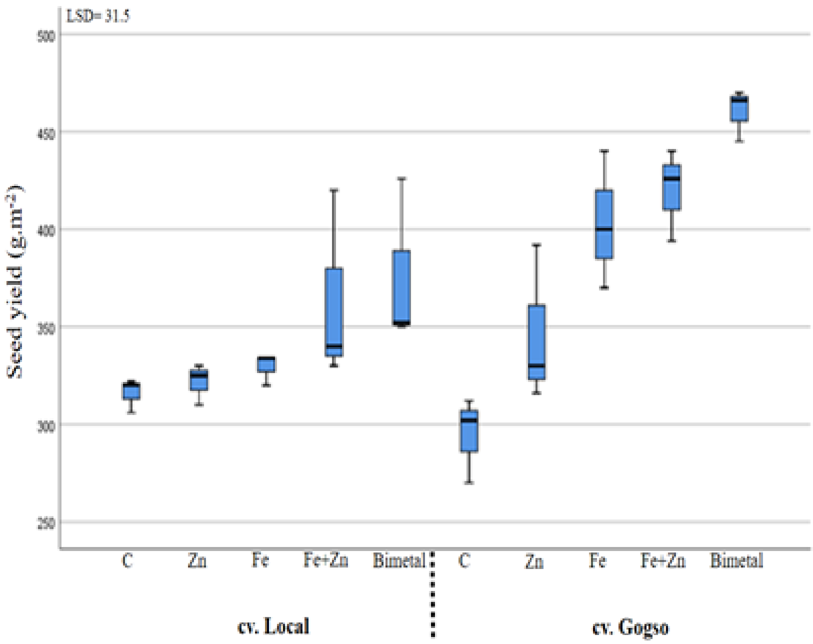

Figure 2. Effects of foliar application of different nano-form micronutrients on seed yield of two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

Fe and Zn foliar application, irrespective to applied source, significantly increased the chlorophyll *a* and *b* and total carotenoids content compared with the control (Table 1). Also, based on the findings it was indicated that the effect of Zn sources on these characteristics was different. The maximum and minimum chlorophyll and carotenoids were detected in foliar sprayed by bimetal nanoparticles and control plants. Carotenoid content was more affected by foliar application than chlorophyll. Our results confirmed other findings regarding significant role of Zn and Fe on leaf pigments content (Janmohammadi *et al.*, 2012; Raliya *et al.*, 2016; Roosta *et al*., 2018).

However, there was no difference in pigment content between cultivars and also the interaction effects of cultivar × foliar application was insignificant. Chlorophyll is one of the major components of chloroplast and is positively related to plant photosynthetic rate. Chlorophyll content could be negatively affected by hot and dry spell at end of spring in Mediterranean region. Thus, a change in chlorophyll contents can directly depict the health of plants as well as plant response to the change in environment. As demonstrated in the results, there was increase in chlorophyll contents in leaves with NPs and it can be resulted in improved photosynthetic parameters and higher seed yield.

Evaluation of APX activity showed that although in both cultivar foliar application of Fe+Zn and bimetal nanoparticles significantly increased the APX activity, cv. Gosgo was more responsive to Fe, Fe+Zn and bimetal nanoparticles than local cultivar (Figure 3). Foliar application of bimetal nanoparticles increased APX activity by 75% and 211% over control in local and Gosgo cultivars, respectively.

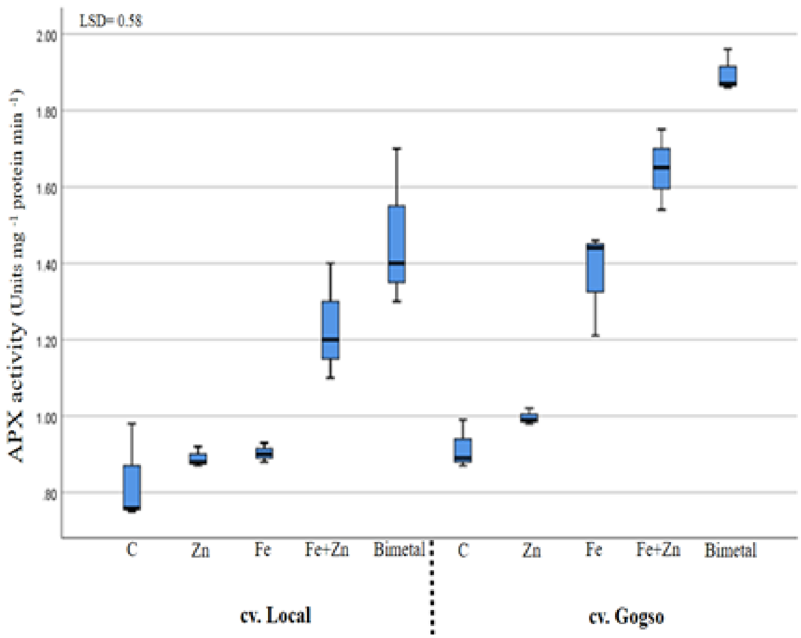

Figure 3. Effects of foliar application of different nano-form micronutrients on ascorbate peroxidase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result ($P \leq 0.05$).

Assessment of CAT activity showed that both micronutrient NPs significantly enhanced the CAT activity .However, the intensity of the effect of iron, zinc and their mixed form in the local cultivar was not very high and cv. Gosgo was more responsive than local cultivar to all nano-particles treatments (Figure 4).

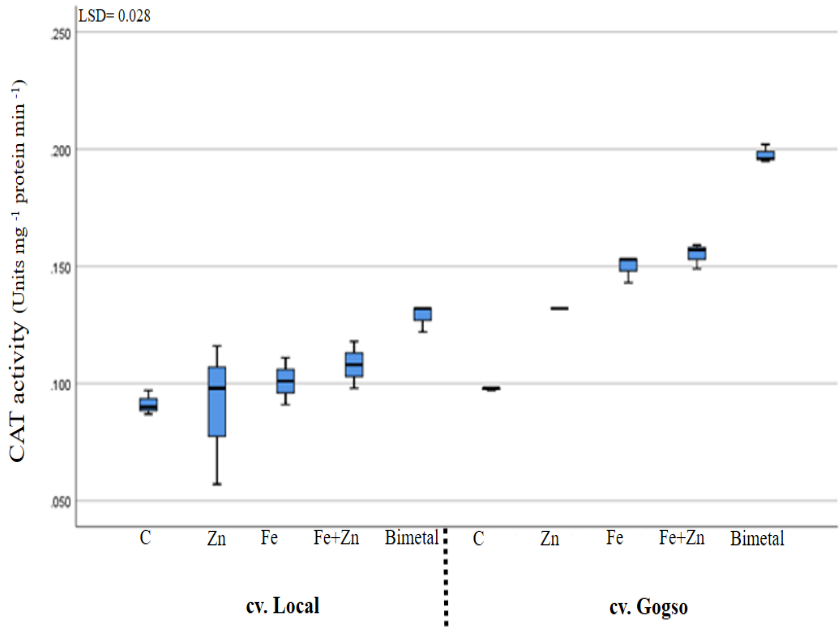

Figure 4. Effects of foliar application of different nano-form micronutrients on catalse activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

The additive effect of bimetal on CAT activity in cv. Gosgo was about 50% higher than the local cultivar. A similar situation was recorded for GPX activity so that the highest activity was recorded for cv. Gosgo sprayed with bimetal, mixed Fe+Zn and Fe (Figure 5). Interestingly, GPX activity in cv. Gosgo under control conditions (without foliar application) was higher than foliar sprayed by monometallic nanoparticles in local cultivar.

Evaluation of SOD activity showed that the positive effects of foliar spray of monometallic nanoparticles was lower than bimetal and mixed Fe+Zn in both cultivars (Figure 6). Although the use of bi-metal significantly increased the activity of this enzyme in the local cultivar, the highest level of activity was recorded in cv. Gosgo with the use of bi-metal and mixed Fe+Zn.

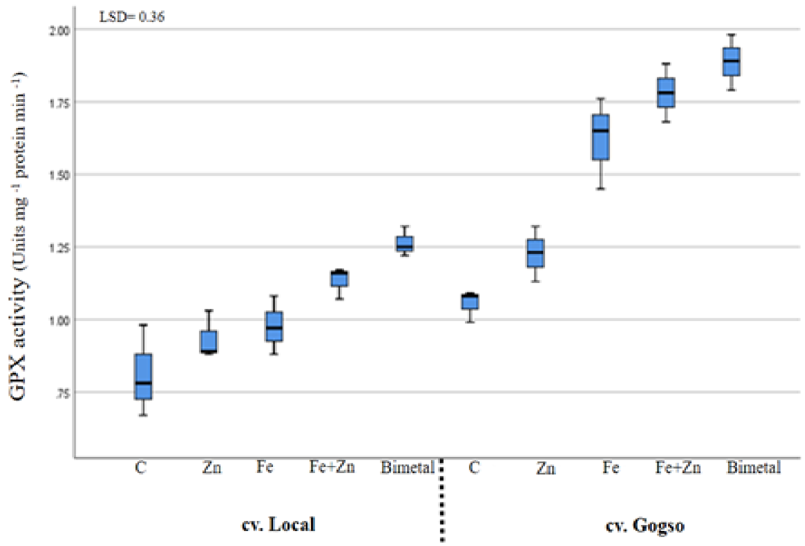

Figure 5. Effects of foliar application of different nano-form micronutrients on guaiacol-peroxidase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

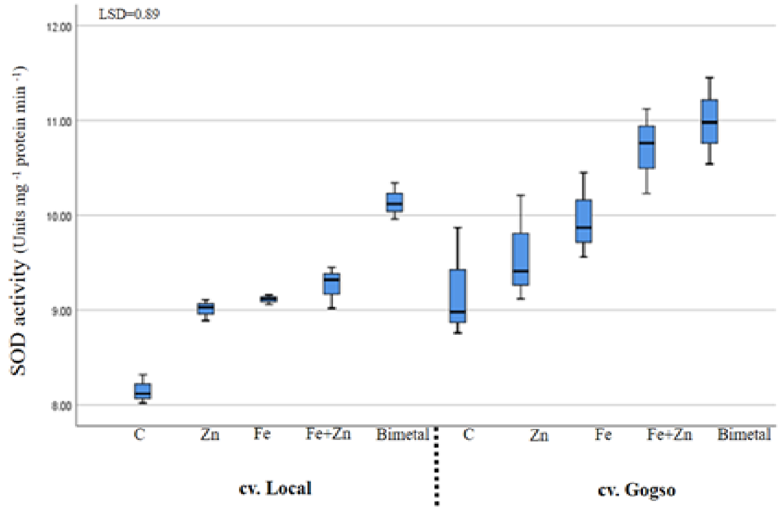

Figure 6. Effects of foliar application of different nano-form micronutrients on superoxide dismutase activity in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

Assessment of H_2O_2 concentration revealed that concentration this reactive oxygen species is very high in local cultivar (Figure 7). Although the use of micronutrients, especially the mixed Fe+Zn and bimetallic nanoparticles, could reduce the concentration H_2O_2 in both cultivars, the percentage of H_2O_2 reduction due to foliar spray of micronutrient was much higher in the local cultivar when compared with cv. Gosgo. This indicates the weakness of antioxidant systems and low capacity of ROS scavenging in local cultivar. The results obtained from the study of H_2O_2 concentration are largely consistent with the results of the antioxidant enzymes activity.

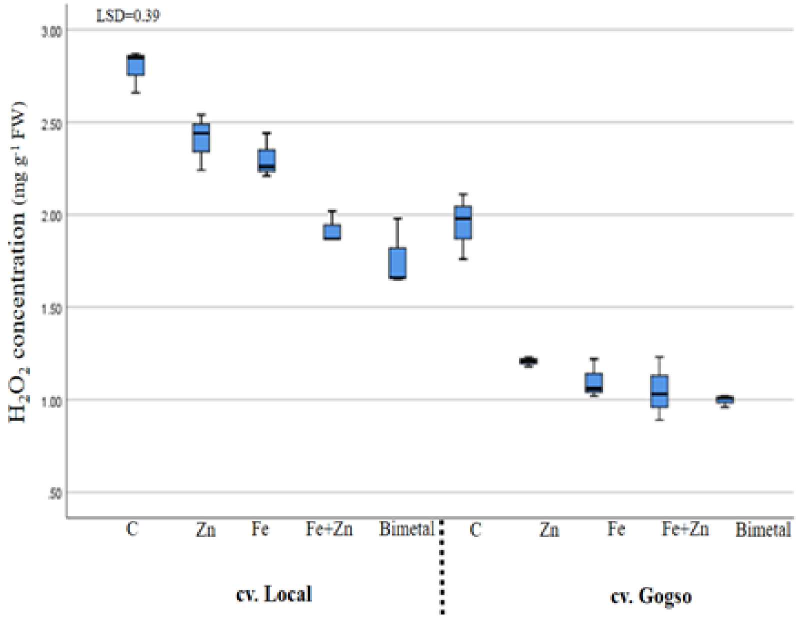

Figure 7. Effects of foliar application of different nano-form micronutrients on H2O2 concentration in two chickpea cultivars (Local and Gogso). C: control (no fertilizer application, Zn: ZnO nanoparticles (Zn-NPs), Fe: Fe3O4 nanoparticles (Fe-NPs), Fe+Zn: mixed Fe3O4+ ZnO nanoparticles, and Bimetal: bimetallic Fe-Zn nanoparticles. Any difference larger than the LSD is considered a significant result (P≤0.05).

It seems that nano-Zn and nano-Fe application, especially in bimetallic form, reduced H2O2 levels, and as a fact, nano-spraying alleviated the adverse effects of abiotic stress. Altogether, application of nano-fertilizers alone is not a totally successful strategy in to improve chickpea production in semi-arid region. A more efficient and sustainable solution to micronutrient deficiency limitations to crop production is the development and use of micronutrient-efficient plant genotypes that can more effectively grow on soil with low phytoavailable micronutrient contents, which would reduce fertilizer inputs and protect the environment. Selection of plant genotypes that can tolerate low nutrient supply may increase productivity on low fertility soils and reduce fertilizer requirements. By selecting suitable genotypes applying a new generation of bi-metallic micronutrient fertilizers can be more successful.

CONCLUSIONS

Finding revealed that both Zn and Fe should be applied in studied site. Study results depicted that separate and individual consumption of both Zn and Fe NPs increased growth and seed yield components in both local and new introduced cultivars. However, results showed that with the combined consumption of these elements extensive positive effects on antioxidant activity, scavenging of reactive oxygen species and pigment content. This indicates the deficiency of both elements in studied site and their synergistic effects in improving growth and physiological processes. Our finding showed that the best plant performance obtained by application of bimetallic Fe-Zn nanoparticles especially in new introduced cultivar. The use of bimetallic fertilizers can be promising in the production of nano composite fertilizers. In bimetallic nanoparticles, two different metals combine to show novel properties which are the combination of the two metals present in it. Despite the fact that some knowledge has been acquired through present and previous studies, many questions still remain unanswered such as the fate and behavior of bimetallic nanoparticles in plant systems and its effects on seed quality and human health.

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