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Evaluation Effect of Different Levels of Zinc and Manganese Nano-Chelate on Quantitative and Qualitative Traits of Cowpea (*Vigna unguiculata* L.)

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ABSTRACT

Nano fertilizers are new products which contain readily available nutrients in the nano scale range and are preferred largely due to their efficiency. This research was conducted to assessment the effect of nano chelated of micro nutrient on agronomic traits of Cowpea via factorial experiment based on randomized complete block design with four replications during 2016 seasonal year. The treatments included zinc nano chelated (5, 10 and 10 kg.ha^{-1}) and manganese nano chelated (0, 4 and 8 kg.ha^{-1}). The results of analysis of variance showed that effect of nano chelated of zinc and manganese had a significant effect on plant height, pod length, yield and its components also interaction effect of treatments on number of pods per m^2, seed yield, biological yield and protein yield was significant. Result of mean comparison revealed the highest plant height (154.54 cm), pod length (13.14 cm), number of pods per m^2 (116.47), number of pods per plant (14.52), number of seeds per pod (11.36), seed weight (23.03) and seed yield (210.08 g.m^{-2}) belonged to 10 kg.ha^{-1} nano zinc chelate zinc treatment and the lowest amount of mentioned traits belonged to control (no fertilizer). Between different level of nano manganese chelate the highest seed yield and its components belonged to 8 kg.ha^{-1} treatment. According result of interaction of treatments indicated the highest number of pods per m^2 (131.6), seed yield (244.7 g.m^{-2}), biological yield (544.5 g.m^{-2}) and protein yield (76.8 g.m^{-2}) belonged to 10 kg.ha^{-1} nano zinc chelate and 8 kg.ha^{-1} nano manganese chelate and the lowest amount of mentioned traits belonged to control (no fertilizer). Finally based on result of this research use 10 kg.ha^{-1} nano zinc chelate and 8 kg.ha^{-1} nano manganese chelate improve quantitative and qualitative yield of cowpea and can be advised to farmers.

Keywords: *Micro elements, Nutrition, Protein, Yield.*

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INTRODUCTION

Cowpea is a quick growing and high yielding crop fed to livestock and also makes a valuable contribution towards human food in tropical and subtropical parts of the world (Kumar *et al.* 2014; Sarma *et al.* 2014). Suitable and useful usage of different kind of fertilizers is the main way for reformation and potential of soil fertility and increasing of crops yield (Talaei, 2012). Addition of fertilizers to supplement the natural soil fertility is essential for modern crop production, and precise management of nutrient elements is essential for a sustainable agriculture production (Barker and Pilbeam, 2006). Each plant needs to certain fertilizers according to its needs and soil analyze results. Also micro-elements are the critical elements for plants; however, microelements play the important role in crop productivity where it is used in low rate. Optimum plant nutrition and maximum yield is achieved when nutrient elements are available for plant during the growing season (Malakooti and Tabataei, 1998). Zinc (Zn) is one of the essential micronutrients for plants, and Zn deficiency is common in many crops (Ojeda-Barrios *et al*., 2014). Zinc is a necessary component of various enzyme systems for energy production, protein synthesis, energy production, maintains the structural integrity of bio membranes and growth regulation (Hansch and Mendel, 2009). Zinc deficiencies are mainly found on sandy soils low in organic matter and on alkaline soils. Uptake of zinc also is adversely affected by high pH, high levels of available phosphorus and iron in soils (Ghasemi-Fasaei and Ronaghi, 2008). Zinc activates enzymes and involved in protein, lipids, carbohydrates and nucleic acid metabolism. Zinc plays an important role as a metal component of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes (Marschner, 1986). Zinc has a major role in cell defenses against ROS and as a protective factor against several chemical compositions of oxidation such as membrane lipids, protein, chlorophyll, and enzyme having SH and DNA (Cakmak, 2000). Zinc is required for the activity of different enzymes, including dehydrogenases, aldolases, iso-merases, trans-phospho rylases, RNA and DNA polymerases, and is also involved in the synthesis of tryptophan, cell division, maintenance of membrane structure and photosynthesis, and acts as a regulatory cofactor in protein synthesis (Marschner, 2012). Zinc is an essential element for plants and animals and plays an important role in plants metabolic system. This element activates enzymes and involved in protein, lipids, carbohydrates and nucleic acid metabolism (Zlatimira and Doncheva, 2002). Nearly 200 enzymes and transcription elements of zinc need it as one of the most essential components. Zinc plays an important role in protein and carbohydrates syntheses. It also has effects on growth of stem and root (Kabata-Pendias and Pendias, 1999). Mental synthetic chelates are recommending according to their stability in soil, solubility in water, absorption capability by plant root or according to soli pH and plant type (Vanluit and Boxma, 1981). Materials that are smaller than 100 nm, at least in one dimension, are generally classified as Nano-materials. Applications of this new technology have been found in agriculture, and Nanotechnologies are already applied to production, processing, storage, packing and transportation of agricultural products (Scott and Chen, 2003; Wiesner *et al*., 2006). Nanotechnology is the developing technology during recent years and operating in all fields of agriculture. Nano micronutrients fertilizers stand out as one of the most useful materials, due to their high efficiency, functionalities, convenient and easy applications (Janmohamadi *et al*., 2016a). Nano-formulated fertilizers can release nutrients more slowly in cooperation with other fertilizers which may lead to improvement of nutrient use efficiency. Application of Nano-fertilizers may improve solubility and dispersion of insoluble nutrients in soil, reduce nutrient immobilization and increase bio-availability (Naderi and Danesh Shahraki, 2013). Many problems in different fields of science and industry have been solved using Nano technologies (Scott and Chen, 2003), which are currently used for production, processing and application of Nano scale complexes. Nano fertilizers can be more efficient, decreasing soil pollution and other environmental risks that may occur when using chemical fertilizers (Naderi *et al*., 2011). One of the advantages of using Nano-fertilizers is that application can be done in smaller amounts than when using common fertilizers (Batsmanova *et al*., 2013; Subramanian *et al*., 2015). Nano-fertilizers can be easily absorbed by crops and may exhibit a prolonged effective duration of nutrient supply in soil/ crop compared to the conventional fertilizers (Rameshaiah and Jpallavi, 2015). Zhang *et al*. (2006) investigated the effects of slow/controlled release fertilizers cemented and coated by nano material on crop. It was found that these Nano composites were safe for wheat seed germination, emergence and growth of seedlings and they can provide a regulated, responsive and on time delivery of nutrients to plants. Also several studies show that exogenous application of some nano particle can significantly improve plant growth (Mandeh *et al*., 2012; Song *et al*., 2013). In the last few years, some researchers tried to examine the potential of nanotechnology to improve fertilizer use efficiency. These efforts led to design and development of Nano fertilizer. Nano technology-based fertilizers could be more soluble or more reactive than their bulk counterparts (Nair *et al*., 2010; De Rosa *et al*., 2010). Foliar application of micro nutrients in semi-arid region can solve the immobilization of element in soil. However, actual movement of Nano particles through the cuticle depends on the nutrient concentration, molecular size, chelating structure, time of application and plant species and environmental condition (Janmohamadi *et al*., 2016a). Given the importance of these elements by improving growth conditions and they can increase yield and its components somewhat. Meanwhile, plant type, variety and concentration of micro-nutrients in the root environment and stages of plant growth can be effective on how to use and how these elements impact on seed quality and quantity. Amounts of some micro nutrients in the environment of root was relatively abundant and soil alkalinity, can be prevent exposure these elements through the soil. However, spraying could have a positive impact on yield and yield components in unsuitable soil pH (Hoseynabadi *et al*., 2006). Mir *et al*. (2015) by evaluate effect of nano and biological fertilizers on carbohydrate and chlorophyll content of forage sorghum reported the highest chlorophyll a (1.59 $mg.g^{-1}$), chlorophyll b (5.31 $mg.g^{-1}$), carotenoid (2.24 $mg.g^{-1}$) and carbohydrate (3.24 $mg.g^{-1}$) were achieved from combine bio fertilizers (Azeto barvar 1 + Phosphor barvar 2) + chelated Nano fertilizers (Fe+K) treatments application. Janmohammadi *et al*. (2016b) reported nano micronutrient fertilizers (iron nano chelate and zinc nano chelate) stand out as one of the most useful materials due to their high efficiency, functionalities, convenient and easy applications. Also, TiO_2 nano particles could increase yield and yield components of barley. Foliar application of nano particles resulted in sustainable and high crop production. Treatment Zinc nano chelate with 2000 ppm nano TiO_2 can be advised to farmers. Tahmasebi and Ranjbar (2003) declared that by increasing amounts of zinc, absorption of nitrogen will be decreased by the plant, yet potassium absorption will be increased. Nanomaterials can be used for designing new Zn fertilizers, with the solubility, diffusion and availability of Zn to plants being affected by the Nano-particulate Zn characteristics (size, specific surface area and reactivity) (Mosannaand Khalilvand, 2015). For instance, positive effects of the application of nano ZnO were reported on seed germination, seedling vigor, leaf chlorophyll content, stem and root growth in peanut (Prasad *et al*., 2012), and the positive effects of nano ZnO contrasted with the negative effects on vegetable seed germination of a bulk form of ZnO (Singh *et al*., 2013). Jokar *et al*. (2015) by evaluate the effects of different levels of iron (0, 0.135, 0.270 and 0.405 mg Fe per kg soil as Fe nano chelate or Fe-EDDHA) on growth parameters, concentration and absorption of Fe and some nutrients in cowpea reported Application of both Fe nano chelate and Fe-EDDHA decreased phosphorus and manganese concentrations in shoots as compared to control treatment. Concentration of zinc and cooper in shoots was not significantly affected by the Fe level. In conclusion, lower rates of Fe nano chelate were required, as compared to Fe-EDDHA, to alleviate iron deficiency in cowpea grown in calcareous soils. This study was conducted to assessment effect of different levels of Zinc and Manganese nano chelate on quantitative and qualitative traits of cowpea.

MATERIALS AND METHODS

Field and Treatment Information

This research was carried out to evaluate Zinc and Manganese nano chelate on seed yield, its components and protein concentration of cowpea via factorial experiment based on randomized complete blocks design with four replications along 2016. Place of research was located in Ahvaz city at longitude 48°40'E and latitude 31°20'N in southwest of Iran. The treatments included zinc Nano-chelate (0, 5 and 10 $kg.ha^{-1}$) and manganese Nano-chelate (0, 4 and 8 $kg.ha^{-1}$). This experiment had 36 plots. Each plot consisted of 6 lines with distance of 50 cm and 5 meters length. The distance between shrubs on every row was 10 cm. The soil characteristics were mentioned in table 1.

Farm Management

Base fertilizers (50 $kg.ha^{-1}$ Nitrogen from urea, 80 $kg.ha^{-1}$ phosphorus from ammonium phosphate and 80 $kg.ha^{-1}$ potassium from potassium sulfate) were added to the soil based on soil tests and the recommendations of the Iranian soil and water research institute at the planting stage.

Table 1. Soil properties of studied field

Soil depth (cm)	**0-15**	**15-30**
Z (ppm)	0.32	0.3
Mg (ppm)	1.4	1.3
Fe (ppm)	9.8	9.54
K (ppm)	120	110
P (ppm)	9.2	8.14
N (ppm)	5.78	4.21
OM (%)	0.702	0.675
pH	7.1	7
EC ($ds.m^{-1}$)	4.5	4
Soil Texture	Clay loam	Clay loam

The light-disk harrow was used to mix the soil and the fertilizer after soil fertilization. The furrower was used to make furrows at a distance of 50 cm. The zinc and manganese Nano-chelate were used in the furrows (with 4cm depth) before planting. The furrows were covered with soil.

Measured Traits

Nitrogen content of the seeds was measured using with the Kjeldahl method (included nitrogen digestion, distillation and titration). The seed protein content was obtained by multiplying the seed nitrogen percent by 6.25. Protein yield was achieved by multiplying protein content by seed yield (Linn and Martin, 1999). After physiological ripening seed yield, biologic yield, pod length, number of pods per m^2, number of pods per plant, number seeds per pod, seed weight and plant height was determined. Harvest index (HI) was calculated according to formula of Gardener *et al*. (1985) as follows:

Equ.1. HI= (Seed yield/Biologic yield) × 100.

Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.8) software and Duncan multiple range test at 5% probability level.

RESULTS AND DISCUSSION

Plant height

According result of analysis of variance effect of zinc and manganese nano chelate on plant height was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). compare different level of zinc nano chelate indicated that maximum plant height (154.54 cm) was noted for 10 $kg.ha^{-1}$ zinc nano chelate and minimum of that (102.57 cm) belonged to control (Table 3).

Table 2. ANOVA result of measured traits

S.O.V	df	Plant height	Pod length	Number of pods per m^2	Number of pods per plant	Number of seeds per pod
Replication	3	2.75 ^{ns}	0.07 ^{ns}	73 ^{ns}	0.494 ^{ns}	0.18 ^{ns}
Nano chelated zinc (Z)	2	284.55*	3.67**	1904**	17.045**	6.02**
Nano chelated manganese (M)	2	214.64*	4.91**	1413**	249.51**	5.33**
Z*M	4	1.65^{ns}	0.02^{ns}	1050.1**	2.73^{ns}	0.1^{ns}
Error	24	34.29	0.38	97.3	3.16	0.52
CV (%)	-	4.44	5.3	9.33	14.45	7.63

ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Continue Table 2. ANOVA result of measured traits

S.O.V	df	Seed weight	Seed yield	Biological yield	Protein yield
Replication	3	17.06 ^{ns}	204.3 ^{ns}	103^{ns}	35.1^{ns}
Nano chelated zinc (Z)	2	85.25**	129792**	70595*	441*
Nano chelated manganese (M)	2	77.06**	112822**	55119*	105*
Z*M	4	6.5^{ns}	7084.6**	3510*	889*
Error	24	6.92	365.7	639	113
CV (%)	-	12.58	10.07	6.5	4.1

ns, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

Number of pods per m^2

According result of analysis of variance effect of zinc and manganese Nano-chelate and interaction effect of treatments on number of pods per m^2 was significant at 1% probability level (Table 2). Assessment mean comparison result indicated in different level of zinc Nano-chelate the maximum number of pods per m^2 (116.47) was noted for 10 $kg.ha^{-1}$ and minimum of that (95.84) belonged to control treatment (Table 3). Compare different level of manganese nano chelate showed that the maximum and the minimum amount of number of pods per m^2 belonged to 8 $kg.ha^{-1}$ (118.3) and control (92.52) treatments (Table 3). Evaluation mean comparison result of interaction effect of treatments indicated maximum number of pods per m^2 (131.6) was noted for 10 $kg.ha^{-1}$ zinc Nano-chelate and 8 $kg.ha^{-1}$ manganese Nano-chelate and lowest one (92) belonged to control treatment (Table 4). Some researchers such as Asodeh (2009) confirmed mentioned result.

As for Duncan classification made with respect to different level of manganese nano chelate maximum and minimum amount of plant height belonged to 8 $kg.ha^{-1}$ (155.3 cm) and control (105.73 cm) (Table 3). Another researcher such as Harssine *et al*. (2014); Moallim and Eshqizade (2007) reported same result.

Pod length

Result of ANOVA showed effect of zinc and manganese nano chelate on pod length was significant at 1% probability level, but interaction effect of treatments was not significant (Table 2). According result of mean comparison maximum of pod length (13.4 cm) was obtained for 10 $kg.ha^{-1}$ zinc Nano-chelate and minimum of that (10.09 cm) was for control treatment (Table 3). Evaluation mean comparison result indicated in different level of manganese Nano-chelate the maximum pod length (13.46 cm) was noted for 8 $kg.ha^{-1}$ and minimum of that (9.86 cm) belonged to control treatment (Table 3), Fatahe (2006) reported same result.

Table 3. Mean comparison effect of different levels of nano chelated zinc and manganese on measured traits

Treatment	Plant height (cm)	Pod length (cm)	Number of pods per m^2	Number of pods per plant	Number of seeds per pod	Seed weight (g)	Seed yield ($g.m^{-2}$)
Nano zinc chelate							
0 (control)	102.57 ^{c}	10.09 ^{c}	95.84 ^{c}	10.21 ^{c}	7.11 ^{c}	18.66 ^{c}	175.17 ^{c}
5 ($kg.ha^{-1}$)	138.18^b	11.61 ^{b}	104.8 ^{b}	12.18 ^{b}	9.86 ^{b}	21.01 ^{b}	184.17 ^{b}
10 ($kg.ha^{-1}$)	154.54 ^{a}	13.14 ^{a}	116.47 ^{a}	14.52 ^{a}	11.36 ^{a}	23.03^a	210.08 ^{a}
Nano zinc manganese							
0 (control)	105.73 ^{c}	9.86 ^{c}	92.52 ^{c}	10.53 ^{c}	7.87 ^{c}	18.94 ^{c}	173.26 ^{c}
4 ($kg.ha^{-1}$)	134.26 ^{b}	11.54 ^{b}	106.28 ^{b}	12.37 ^{b}	9.31 ^{b}	20.88 ^{b}	187.55 ^{b}
8 ($kg.ha^{-1}$)	155.3 ^{a}	13.46 ^{a}	118.3 ^{a}	14 ^{a}	11.15 ^{a}	22.9 ^{a}	208.61 ^{a}

*Similar letters in each column show non-significant difference at 5% probability level in Duncan's multiple rang test.

Number of pods per plant

Result of analysis of variance showed effect of zinc and manganese Nano-chelate on number of pods per plant was significant at 1% probability level, but interaction effect of treatments was not significant (Table 2). Evaluation mean comparison result revealed in different level of zinc Nano-chelate the maximum number of pods per plant (14.52) was noted for 10 $kg.ha^{-1}$ and minimum of that (10.21) belonged to control treatment (Table 3). Between different levels of manganese Nano-chelate the maximum number of pods per plant (14) was observed in 8 $kg.ha^{-1}$ and the lowest one (10.53) was found in control treatment (Table 3). Shojaei and Makariyan (2015) by evaluate the effect of three levels of zinc fertilizers (control, 5, 10 g per liter of zinc oxide) on yield and its components of Mungbean reported that zinc fertilizer significantly increased the number of pods per plant.

Number of seeds per pod

According result of analysis of variance effect of zinc and manganese nano chelate on number of seeds per pod was significant at 1% probability level but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of zinc Nano-chelate indicated the maximum and the minimum amount of number of seeds per pod belonged to 10 $kg.ha^{-1}$ (11.36) and control treatment (7.11) (Table 3). Among different level of manganese Nano-chelate maximum number of seeds per pod (11.15) was obtained for 8 $kg.ha^{-1}$ and minimum of that (7.87) was for control treatment (Table 3). Asodeh (2009); Fatahe (2006); Foadi (2014) and Saeedin (2016) reported same result.

Table 4. Mean comparison interaction effect of different levels of nano chelated zinc and manganese on measured traits

Nano-zinc chelate	Nano zinc manganese	Number of pods per m^2	Seed yield ($g.m^{-2}$)	Biological yield ($g.m^{-2}$)	Protein yield ($g.m^{-2}$)
	0	92^{e}	166.5^{e}	420.6^{f}	39.9^{d}
Control	4 ($kg.ha^{-1}$)	98^{ed}	174.6^{d}	435^{e}	47.1^{c}
	8 ($kg.ha^{-1}$)	100^{d}	184.4^{c}	490.3^{cd}	55.3^{c}
	0	95.6^{ed}	169.3^{e}	429.6^{ef}	42.3^{cd}
5 ($kg.ha^{-1}$)	4 ($kg.ha^{-1}$)	102^{d}	186.5^{cd}	484^{d}	52.2^{c}
	8 ($kg.ha^{-1}$)	113.3^{c}	196.7^{b}	499^{c}	59^{b}
	0	106.6^{cd}	183.9^{c}	501^{c}	47.8^{c}
10 ($kg.ha^{-1}$)	4 ($kg.ha^{-1}$)	121^{b}	201.7^{b}	515.5^{b}	58.4^{b}
	8 ($kg.ha^{-1}$)	131.6^{a}	244.7^{a}	544.5^{a}	76.8^{a}

*Similar letters in each column show non-significant difference at 5% probability level in Duncan's multiple rang test.

Seed weight

According result of ANOVA effect of zinc and manganese Nano-chelate on seed weight was significant at 1% probability level, but interaction effect of treatments was not significant (Table 2). Mean comparison result of different level of zinc Nano-chelate indicated the maximum seed weight (210.08 gr) was obtained for 10 $kg.ha^{-1}$ and minimum of that (175.17 gr) was for control treatment (Table 3). Compare different level of manganese nano chelate showed that the maximum and the minimum amount of seed weight belonged to 8 $kg.ha^{-1}$ (22.9 gr) and control (18.94 gr) treatments (Table 3).

Seed yield

Result of analysis of variance revealed effect of zinc and manganese nano chelate and interaction effect of treatments on seed yield was significant at 1% probability level (Table 2). According mean comparison result of different level of zinc Nano-chelate the maximum seed yield (210.08 $gr.m^{-2}$) was observed in 10 $kg.ha^{-1}$ and the lowest one (175.17 $gr.m^{-2}$) was found in control treatments (Table 3). Between different levels of manganese Nano-chelate highest value of seed yield was belonged to the 8 $kg.ha^{-1}$ treatment (208.61 $gr.m^{-2}$) and the lowest one was found in the control treatment as 173.26

$gr.m^{-2}$ (Table 3). Evaluation mean comparison result of interaction effect of treatments indicated maximum seed yield (244.7 $gr.m^{-2}$) was noted for 10 $kg.ha^{-1}$ zinc nano chelate and 8 $kg.ha^{-1}$ manganese nano chelate and lowest one (166.5 $gr.m^{-2}$) belonged to control treatment (Table 4). Bean yield is a complex and quantitative trait and its components are, pod number, seeds number in pod and seed weight (Padilla Ramirez *et al*, 2005). Sheykh baglou *et al.* (2010) showed that application of Nano fertilizers such as nano iron oxide particles increased soybean yield. Farnia and Omidi (2015) by evaluate the effect of nano Zinc chelate and nano bio-fertilizer on yield and yield components of maize under water stress condition reported application of Zn nano fertilizer and nano bio-fertilizer treatment had the highest and control treatment had the lowest biomass yield. Also, 7 day irrigation period treatment with use of nano bio-fertilizer had the highest number of row per cob, number of seed per cob and seed yield but 21 day irrigation period treatment without use of nano bio-fertilizer had the lowest of them. However, 7 day irrigation period treatment with use of Zn nano fertilizer had the highest seed yield and non application of Zn nano fertilizer in 21 day irrigation period treatment had the lowest of seed yield.

Biological yield

According result of analysis of variance effect of zinc and manganese nano chelate and interaction effect of treatments on biological yield was significant at 5% probability level (Table 2). Assessment mean comparison result of interaction effect of treatments showed maximum biological yield (544.5 $gr.m^{-2}$) was noted for 10 $kg.ha^{-1}$ zinc nano chelate and 8 $kg.ha^{-1}$ manganese nano chelate and lowest one (420.6 $gr.m^{-2}$) belonged to control treatment (Table 4). Micronutrients improve plant growth, which matter is probably due to improved carbon uptake, increased synthesis of metabolites and maintenance of the plant water status. These results were consistent with findings of this study. Biological yield increased by application of 10 $kg.ha^{-1}$ zinc fertilizer due to increased production of dry matter in cowpeas and more accumulation of dry matter at the end of this period. On the other hand, increase in dry matter due to application of zinc element can enhance biosynthesis of auxin, increase chlorophyll content and enhance enzymatic activity of phospho enol pyruvate carboxylase and ribulose bis phosphate carboxylase, decrease sodium accumulation in plant tissues and increase uptake of nitrogen and phosphorus (Saeedi Abou eshaghi and Yidwi, 2015). Saeedin (2016) evaluated the correlation between biological yield and seed yield of cowpea and reported a positive and significant correlation between mentioned traits. Its seem biological yield increased because of accumulation of photosynthetic products (source products) and high potential of seeds (reservoir) for absorption and accumulation of dry matter. Therefore, any increases in seed yield also increases the biological yield. However, less dry matter is accumulated in case of micronutrient deficiency, which decreases the biological yield. Soleymani and Shahrajabian (2016) reported that application of zinc fertilizer treatment led to the highest biological yield and seed yield due to the positive effect of zinc on biosynthesis of auxin and positive effect of iron on photosynthesis.

Protein yield

Result of analysis of variance revealed effect of zinc and manganese Nano-chelate and interaction effect of treatments on protein yield was significant at 5% probability level (Table 2). Evaluation mean comparison result of interaction effect of treatments indicated maximum protein yield (76.8 $gr.m^{-2}$) was noted for 10 $kg.ha^{-1}$ zinc nano chelate and 8 $kg.ha^{-1}$ manganese nano chelate and lowest one (39.9 $gr.m^{-2}$) belonged to control treatment (Table 4). Reduced protein content in case of zinc deficiency seems to decrease RNA levels and the amount of ribosome in the cells. Reduced synthesis of RNA restricts synthesis of protein while glucose, non-protein nitrogen and DNA levels increase in the cells. Zinc is also involved in dehydrogenase and protease activity and has a key role in protein synthesis (Marshner, 1995). Foadi (2014) also showed that application of zinc sulfate increased protein content of the seeds. These results were consistent with the results of this study. In this regard, Mabhot (2017) reported that nano chelate zinc with large specific surface area, high solubility and a certain complex is highly absorbed by plants. This increases photosynthesis capacity of the plant and allocates more photosynthetic products to reproductive organs and increases seed yield, which directly or indirectly increases protein content of the seeds. Marschner (1993) reported that, by increasing consumption of Iron and zinc in corn, we can witness that the total amount of carbohydrate and seed protein is increased, and as a result the gain weight, number of seeds and at last yield will be increased as well. Bakhash Klarastaghi *et al.* (2007) also showed that application of zinc fertilizer relatively increased seed yield and protein content of the seed. These results were consistent with the results of this study. Protein percentage increases by application of micronutrients since these elements (especially manganese) are involved in metabolism of nitrogen and synthesis of enzymes. These elements also regulate enzymatic activity of a large number of enzymes. Enzymes are synthesized from protein (Jamshidi *et al.*, 2016). Sarbandi and Madani (2014) reported that application of micronutrients (manganese and zinc) significantly improved yield, yield components, biological yield and protein percentage, the highest protein percentage belonged to micro fertilizer treatment and the lowest protein percentage belonged to control. Jalilshesh Bahre (2014) reported that zinc and manganese significantly increased protein content of the seeds. These elements constitutes the structure of some enzymes, are involved in synthesis of amino acids, which are essential in synthesis of proteins. Therefore, application of these elements (manganese) increases protein content of the seeds. These results were consistent with the results of this study. Jafardoukht *et al.* (2015) showed that application of zinc increased the protein yield that indirectly increased absorption of nitrogen. In other words, micronutrients are available to plants at flowering stage. Therefore, the plants can use nitrogen content of the soil more efficiently. This enhances protein synthesis. These results were consistent with the findings of this study.

CONCLUSION

Generally the results of this experiment showed that different levels of zinc and manganese nano chelated were effective on seed yield, biological yield and protein yield. Finally based on result of this research use 10 $kg.ha^{-1}$ nano zinc chelate and 8 $kg.ha^{-1}$ nano manganese chelate improve quantitative and qualitative yield of cowpea and can be advised to farmers.

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