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Effect of Pluramin and Iron and Zinc nano-fertilizer on rainfed chickpea (*Cicer arietinum* L.) at on-farm conditions

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ABSTRACT

This experiment was conducted to study the effect of pluramin and iron and zinc nanofertilizers on rainfed chickpea in autumn sowing at farmer conditions. The field located next to synoptic weathering station-Varmahang in Kamyaran-Iran during 2015-2016 growing season. The experiment layout was split plots in a randomized complete block design with three replications. The main factor were three levels of pluramin amino acid application (0, 150 g/ha and 250 g/ha) and subplot were four levels of nano fertilizers application (control: distilled water, nano-iron, nano-zinc, and nano-iron + zinc). The results showed that characters as: number of secondary branches, number of pods, number of seeds per pod, 100 seed weight, grain yield, biomass, protein percent and protein yield were significantly affected by the application of pluramin. An application of pluramin significantly increased all traits with exception of protein percent. In this experiment, the application of micronutrients nano-fertilizers also showed significant differences on the number of primary and secondary branches, number of pods, 100 seed weight, grain yield, biomass and protein yield, as this characters were enhanced by nano-fertilizer. Finally, mutual effect of pluramin and nanofertilizers indicated that the most pod numbers, grain yield and biomass were achieved by 250 g pluramin and iron+zinc nano-fertilizers.

Keywords: amino acid, biomass, chickpea, nano-fertilizer, nutrient, protein, rainfed, yield

INTRODUCTION

Chickpea as valuable source of protein, carbohydrates, fat, minerals, B vitamins (Varshney et al., 2017) and Ca, Fe, niacin and vitamin C (Kumawat and Kuldeep, 2017), is favorable rainfed crop under semiarid condition of Iran, where the plant exposed to high temperature and water shortages during reproductive development (Kanouni et al, 2018). Among the over 50 chickpea producers in the world, Iran ranked as third with 5% of total world chickpea production (Varshney et al., 2017), but its average yield (409.6 kg/ha) is very low (FAOSTAT, 2016), Therefore, the significant amount of this plant is imported annually, according to the country's requirements (Varshney et al., 2017). In the other hand, application of permanent macronutrients fertilizer in conjunction with high alkalinity of the soils in semi-arid regions of Iran, enforced low micronutrients availability (Ryan et al., 2012) and consequently lost crop yield (Souri et al., 2018).

Disastrous effects of conventional fertilizers such as: low use efficiency, low productivity and environmental pollution were excitation reason to release new alternative fertilizers, namely nano-fertilizers (Ram et al., 2018). Derosa et al. (2010), Valizadeh and Milic (2016) and Rameshraddy et al. (2017) announced that nano-fertilizers, have some special characters as: large surface area and small size (Less than 100 nm) that help them penetrate to cells and improve availability of nutrients for crop productivity. Nano-fertilizers can enhance crop growth and yield, accelerate seed germination, improve plant establishment, enhance production, booster plant protection system and ameliorative environmental risk (Janmohammadi et al., 2016b; Ram et al., 2018). In order to improving the low productivity of crop, application of micronutrients nano-fertilizers were recommended by some researchers (Mahajan et al., 2011; Tarafdar et al., 2014; Janmohammadi et al., 2016a; Valizadeh and Milic, 2016; Rameshraddy et al., 2017; Ram et al., 2018).

Zinc and iron shortage were accounted as most distinguished yield-limiting factors in the semi-arid region (Ryan, 2008; Kumawat and Kuldeep, 2017; Souri et al., 2018). Various functions of zinc was mentioned as, synthesis of different enzymes and proteins, auxin, pollen formation (Valizadeh and Milic, 2016), protein synthesis (Hänsch and Mendel, 2009), chlorophyll and growth hormones formation (Kumawat and Kuldeep, 2017). Also iron increased synthesis of chlorophyll and enzymes (Valizadeh and Milic, 2016), photosynthesis function, mitochondrial respiration, biosynthesis of some hormones (ethylene, gibberellin acid, jasmonic acid), strengthening the plant defense system (Hänsch and Mendel, 2009), synthesis of nitrogenase, which is necessary for nitrogen fixation in pulses (Kumawat and Kuldeep, 2017).

In different study well detailed the zinc nono-fertilizer function such as: increasing seed germination, seedling vigor and higher leaf chlorophyll content in groundnut (Prasad et al., 2012), accelerated growth of mung and gram seedlings (Mahajan et al., 2011), higher germination percent, higher plant height, number of tillers, chlorophyll content and increased yield of finger millet (Rameshraddy et al., 2017), improved shoot length, root length, root area, chlorophyll content, total soluble leaf protein, biomass, and grain yield of pearl millet (Tarafdar et al., 2014), increasing yield and yield components of maize (Farnia and Omidi, 2015). As well as zinc, iron nano-fertilizer introduced as chlorophyll formation stimulator in Faba bean (Nadi et al., 2013).

In addition to the importance of using nano-fertilizers, in order to improvement crop yield in semi-arid region, application of amino acids was introduced as a promising tool in agroecosystems which is in consistence with an environment. Amino acids as stimulant plant growth, could ameliorative destructive effect of abiotic stresses (Kowalczyk and Zielony, 2008). The various roles of amino acids in plant were documented in literature such as: protein synthesis, the formation of plant tissues and chlorophyll synthesis (Kowalczyk and Zielony, 2008), producing of metabolites and precursors which serve in plant defense system, biosynthesis of vitamin, nucleotide and hormones (Coruzzi and Last, 2000), increasing of antioxidant capacity, improving of the crop uniformity, minimizing nitrate content in plants (Tsouvaltzis et al., 2014), greater activity of the enzymes peroxidase, phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (Teixeira et al., 2017) and increasing of technological characteristics of grain (Popko et al., 2018).

Therefore, in order to increase the yield of rainfed chickpea in semi-arid conditions, this experiment was conducted by application of pluramin amino acids with zinc and iron nano-fertilizers.

MATERIAL AND METHODS

This experiment was carried out as on-farm conditions next to synoptic meteorological site of Varmahang in Kamyaran-Northwest of Iran during 2015-2016 growing season. The geographic coordinates of the tested farm included as 34°47′ N and 46°53′ E, 1,425 m elevation. Experiments were carried out as split plot design with randomized complete block design with three replications.

The main factor were three levels of pluramin amino acid application (0, 150 g/ha and 250 g/ha) and subplot were four levels of nano-fertilizers application (control: distilled water, nano-iron, nano-zinc, and nano-iron+zinc). The meteorological parameters were obtained from a synoptic station located 100 meters far from the field of study during the experiment year (Table 1).

In order to know the some physical and chemical characteristics of the soil, several soil samples were taken from the soil randomly and analyzed by the lab (Table 2). Then, in order to prepare the planting bed, plow and disk were used to cut the husk and to smooth the soil condition of the farm. Chickpea cultivar (Hashem) was sown by planter on November 8, 2015. The spacing

Table 1. Meteorological parameters in synoptic meteorological site during 2015-2016 growing season

Month/Parameters	Minimum temperature (°C)	Maximum temperature (°C)	Minimum relative humidity (%)	Maximum relative humidity (%)	Monthly rainfall (mm)
October	8.8	25.3	27	62.4	68.3
November	-2.2	10	46.3	88.1	12.8
December	1.3	12.1	54	90.8	69.2
January	-2.7	9.3	45.7	87.3	13.6
February	-0.9	13	35.7	76.8	39.7
March	-1.2	13.6	53.3	75	30.4
April	1.8	17.5	32.8	79.8	50.6
May	5.8	26.5	16	60.3	3.3
June	11.5	33.5	12	46.4	0.2
July	17	38.4	9.9	35	0

between rows was 75 cm (to facilitate weed control by cultivator), the planting depth was 6-8 cm and the plant spacing was 7 cm.

During the growing season, weeds grown between plants on the ridges, were removed several times by hand. In order to prevent the pest of heliotypes, Malathion insecticide was sprayed in a ratio of 2/1,000, in two growth staged, first at flowering stage and the other at the beginning of the pod filling stage. The iron and zinc nanofertilizers made by Sepehr Parmys and pluramin amino acid (92% of amino acids) made in Italy (Table 3), were applied as foliar application according to the manufacturer's instructions, using a 20-liter sprayer in two stages, before flowering (May 5) and after flowering (May 30). The harvest took place on July 1, when 80% of the pods were in full-ripening stage. Five plants were selected randomly from each plot in middle-row and were used to measure and record the traits (numbers of secondary branch, pod number, seed per pod, 100 seed weight, grain yield, biomass, protein % and protein yield). To determine the grain yield in each plot, after removing the margins effects, two middle-row harvested, the seeds were separated from the rest of the plant and, using a digital scale, the total weight of the grains harvested was calculated in each plot and after adding the seed weight of 5 plants (selected to determine the yield components), the final weight was recorded as grain yield in the harvested area and then converted to kg per hectare.

Table 2. Physical and chemical characters of soil test in the field of study

Soil texture	EC (ds/m)	pH	OM (%)	NT (%)	P_2O_5 (mg/kg)	K_2O (mg/kg)
Loam	1.8	7.9	1.2	0.13	9.34	185

EC - electrical conductivity as decisiemens per meter, OM - organic matter, NT - total nitrogen.

The percentage of protein was measured using a fully automatic Kjeldahl set (D-40599 model manufactured by Behr- Germany). Protein yield was also obtained by multiplying seed yield in protein percentage. Finally data analysis was performed with SAS $_{(9.1)}$ software and comparison of averages were carried out by Duncan test at 5% probability level.

Table 3. Aminoacid components (Pluramin ™)

Composition	Content
Total nitrogen (N)	14% w/w
Organic nitrogen (N) soluble in water	13.8% w/w
Ammonic nitrogen (N)	0.2% w/w
Organic matter	92% w/w
Total aminoacids	90% w/w

RESULTS AND DISCUSSION

Primary and secondary branch

The results of the analysis of variance showed that pluramin had a significant effect on secondary branches (Table 4). In this study, pluramin could increase secondary branches in chickpea. As shown in Table 5, the maximum secondary branches were achieved by 250 g pluramin. It seems that amino acid could enhance photosynthetic pigments and NPK uptakes in this study as mentioned by Hammad and Ali (2014) about wheat. The results of this study coincided with other previous research. Increased plant height and number of branches by foliar application of amino acids were reported by EL-Zefzafy et al. (2016). In another study, plant height, number of leaves and stems were increased significantly with high concentration of amino acids (Shafeek et al., 2016).

Improving secondary branch in this study may be attributed to increasing gibberllic acid and indole acetic acid by amino acid spraying that reported by Talaat et al. (2005) or increasing cell division, cell enlargement and consequently growth parameters that were found by Shekari and Javanmardi (2017). Improved leaf area, shoot dry weight and nutrition concentrations in leaves and pods could increase significantly when plants treated by amino acids foliar applications (Shekari and Javanmardi, 2017; Souri et al., 2018).

In this study nano-fertilizer treatment had significant effect on primary and secondary branches numbers at the level of 0.05% (Table 4), as they enhanced by nano-fertilizers (Table 5). In the similar study, Nadi et al. (2013) were found the highest chlorophyll content by nano-iron. They announced that iron micronutrient have some functions in plant as chlorophyll formation, photosynthesis, enzyme systems, respiration, increasing of the yield performance. Also Prasad et al. (2012) recorded increased in stem and root growth by Zn nanoparticles. In another study, Zn nano-fertilizer affected the growth of mung and gram seedlings (Mahajan et al, 2011).

Rameshraddy et al. (2017) concluded that Zinc nano-fertilizer had some favorable effects on finger millet, such as: improved germination percent, root and shoot, plant height, number of tillers and chlorophyll content.

Yield components

Pluramin had significant effects on pod numbers, seed per pods and 100 seed weight. On the mean comparison data in Table 5, these characters were increased by pluramin. Shafeek et al. (2016) demonstrated that high concentration of amino acids could enhance pod length, pod wide, pod weight, number of pods per plant and weight of 100 seeds.

It seems that improve conditions such as: relative water content, photosynthetic pigments, total soluble sugars, total carbohydrates, total free amino acids, enzymes activities, minerals uptakes could be an increasing factor of yield components, as reported by Hammad and Ali (2014) following amino acid application on wheat during a two-year study. Also in another study augmentation of pod yield were higher significantly when plants treated by aminoacids foliar applications (Souri et al., 2018).

In this study, pod numbers and 100 seed weight also affected by the nano-fertilizer foliar application. As found in table 5 the maximum pod numbers observed by all nano-fertilizer treatments and the most seed weight were obtained by Fe+Zn nono-fertilizers.

The application of pluramin and nano-fertilizers increased the number of pods compared to the control. So that the maximum number of pods was obtained in the treatment of 250 g of pluramin and nano-iron+zinc (Figure 1).

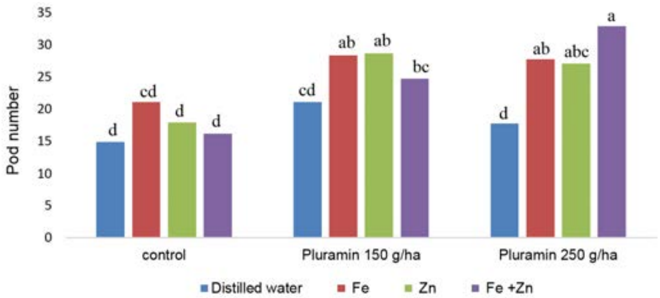

Figure 1. Interaction effects of pluramin and nano-fertilizers on number of pods per plant ([a, b, c, d] Different letters above columns were significantly different from each other at P≤0.05 according to Duncan's multiple range test.)

Table 4. Anova analyses for pluramin and iron and zinc nano-fertilizer on rainfed chickpea

SOV	df	Numbers of primary branch	Numbers of secondary branch	Pod number	Seed per pod	100 Seed weight	Grain yield	Biomass	Protein	Protein yield
Block	2	0.03ns	8.59ns	8.78ns	0.009ns	2.34ns	53,260.97ns	147,307.16ns	3.73ns	4,702.48ns
Pluramin	2	0.19ns	22.12*	292.47**	0.01*	39.1**	1,405,254.79**	5,156,427.11**	14.92*	70,387.18**
Ea	4	0.16	4.46	8.25	0.01	2.23	61,057.18	207,818.73	2.18	3,417.52
Nano-Fertilizer	3	0.33*	16.34*	114.91**	0.001ns	4.79*	354,098.43**	1,403,936.28**	0.88ns	22,386.91**
P × Nano-F	6	0.1ns	3.19ns	32.38*	0.001ns	3.45ns	91,762.28*	420,165.44*	3.61ns	3,658.67ns
Eb	18	0.09	4.03	10.96	0.001	2.1	33,935.75	154,906.72	1.9	2,662.19
CV (%)		10.04	17.04	14.31	4.38	5.19	15.36	16.32	5.48	17.31

**, *Significant at 1% and 5% level an arrangement, ns non-significant. SOV - sources of variation, CV - coefficient of variation.

Table 5. Mean comparison of pluramin and iron and zinc nano-fertilizer on rainfed chickpea

Treatment\ characters	Numbers of primary branch	Numbers of secondary branch	Pod number	Seed per pod	100 Seed weight	Grain yield (kg/ha)	Biomass (kg/ha)	Protein (%)	Protein yield (kg/ha)
Pluramin:									
0	2.84a	10.26b	17.44b	0.95b	25.19b	803.97b	1,656.8b	26.35a	209.6b
150 g/ha	3.01a	12.23a	25.65a	1.02a	28.08a	1,404.13a	2,843.5a	24.19b	341.34a
250 g/ha	3.08a	12.87a	26.29a	0.96b	28.51a	1,388.95a	2,732.6a	24.81b	343.15a
Nano-Fertilizer:									
Control: distilled water	2.7b	9.87b	17.83b	0.99a	26.21b	904.06b	1,827.1b	25.44a	224.94b
Fe	3.07a	12.15a	25.66a	0.97a	27.48ab	1,324.91a	2,632.6a	25.24a	335.21a
Zn	3.13a	12.1a	24.48a	0.97a	27.46ab	1,262.45a	2,513.5a	24.7a	309.18a
Fe + Zn	3.01a	13.03a	24.53a	0.98a	27.89a	1,304.64a	2,670.6a	25.08a	322.79a

a,b Mean with the common letters in each column have not significant differences at 0.05 probability level by Duncan’s multiple range test.

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Janmohammadi et al. (2016b) announced that the maximum numbers of tuber per plant, mean tuber weight and tuber weight per plant were obtained by application of complete nano-fertilizer and nanochelated Zn+B.

In another study, foliar application of the Fe and Zn nano-fertilizers enhanced seed number up to 11% and 13%, respectively, in compared to control (Janmohammadi et al., 2016a). Also, Prasad et al. (2012) were found an increasing pod yield as well as 34% by Zn nanoparticles.

Grain yield and biomass

Grain yield and biomass increased significantly by pluramin. Considering the increase in the number of secondary branches and yield components of chickpea in the previous part, by this treatment, the occurrence of such result seems logical.

Agrawal et al. (2018) found significant and positive correlation of grain yield with number of primary and secondary branches, biological yield, harvest index, 100 seed weight and days to maturity. Atta et al. (2008) reported that number of secondary branches, pods per plant and seed size are major yield contributing factors in chickpea cultivars. Also Hagos et al. (2018) were found that seeds per plant, biomass, days to maturity and 100 seed weight had positive effect on improving seed yield.

Improving uptake and transportation of micronutrients inside the plant due to chelating effect of amino acids on micronutrients were reported by Ibrahim et al. (2007).

Also increasing the biomass and crop yield were reported by foliar application of amino acids (EL-Zefzafy et al., 2016).

The great efficacy of amino acids foliar application were proven by some researchers, for example increasing yield and its components of broad bean seeds (Shafeek et al., 2016), improvement of yield and its attributes and also reduce hazards of drought stress of wheat (Hammad and Ali, 2014), improvement of chemical fruit properties and yield of squash (Abd El-Aal et al., 2010), higher pod yield of bean (Souri et al., 2018), increased fresh and dry biomass of broccoli (Shekari and Javanmardi, 2017) and increasing of grain yield of winter wheat (Popko et al., 2018).

In this study, also grain yield and biomass increased significantly by nano-fertilizers. Finally, interaction effects of pluramin and nano-fertilizers on grain yield and biomass were different significantly (Figure 2 and 3). As shown the highest grain yield and biomass were achieved by 250 g pluramin and Fe+Zn nano-fertilisers. Since, the maximum number of pods was obtained in the same treatment and considering the importance of this trait as one of the important components of the yield, the occurrence of such result, seems logical.

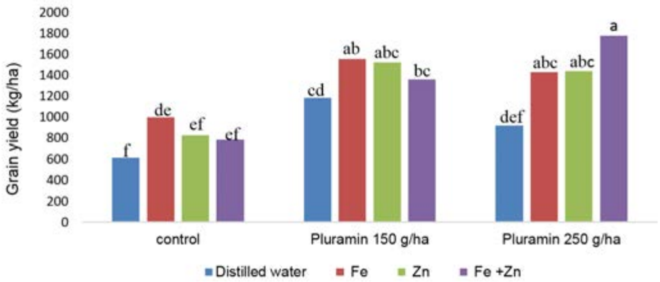

Figure 2. Interaction effects of pluramin and nano-fertilizers on grain yield ([a, b, c, d, e, f] Different letters above columns were significantly different from each other at P≤0.05 according to Duncan's multiple range test.)

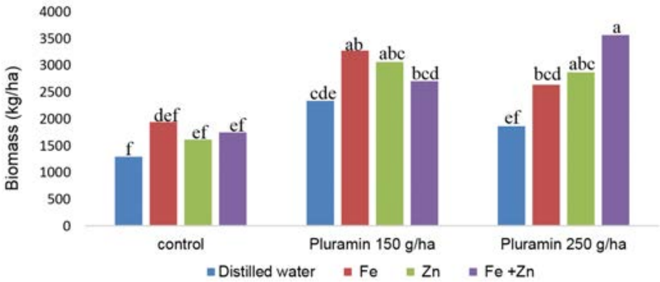

Figure 3. Interaction effects of pluramin and nano-fertilizers on biomass ([a, b, c, d, e, f] Different letters above columns were significantly different from each other at P≤0.05 according to Duncan's multiple range test.)

Improving of yield and its components by foliar spray of both amino acids and micronutrients declared by Khalil et al. (2008). Also, Janmohammadi et al. (2016a) decleard the highest potato tuber yield by complete nano-fertilizer and nanochelated Zinc+Boron. In other research, foliage application of nano-fertilzer could increase the absorption of necessary nutrient by plants (Valizadeh and Milic, 2016).

In addition to, enhancing the water use efficiency and improving the productivity of maize were reported in semi-arid regions by nano-fertilizers (Janmohammadi et al., 2016b). Rameshraddy et al. (2017) were found an increase about 21.4% in finger millet yield by Zn nano-fertilizer. They concluded that achieving the best performance was due to increasing chlorophyll, relative water content and less percent membrane leakage.

Janmohammadi et al. (2016b) demonstrated that nano-fertilizer can be an efficient nutrient management strategy in semi-arid regions. They observed the highest spike length, number of the grains per spike, chlorophyll content, and grain yield of barley by application of nano-fertilizer. Efficacy of zinc nano-fertilizer was superior to iron, as zinc nano-fertilizer increased the seed yield more than 6% over to control. They also reported prolonged course length to anthesis and maturity by iron and zinc nano-fertilizers, and finally concluded that increasing seed yield was due to increasing assimilate supply, levels of cytokines and sink size.

Due to the importance of zinc and iron elements in physiological and biochemical reactions, increasing leaf growth and straw yield was predictable (Janmohammadi et al., 2016a). Also Tarafdar et al. (2014) suggested that application of zinc nano-fertilizer on pearl millet significantly improved plant dry biomass, and increased the grain yield by 37.7%.

Protein

Surprisingly, the percentage of protein decreased significantly by the pluramin amino acid (Table 4 and 5). It seems that due to the increase in grain yield and the negative relationship between grain yield and protein percentage, such a results has been made. Gaur et al. (2016) were observed that the protein content was negatively correlated with seed size and grain yield. They concluded that, an enhanced in protein percent had a negative effect on seed size and grain yield. Also, negative correlation between seed yield and protein were found by other researchers (Tayyar et al., 2008; Rahimi Azar et al., 2013).

Coruzzi and Last (2000) concluded that the amino acids effects varied based on different plant growth stages, especially critical stages of growth. In this study it was probable that an increase in protein content would occur if another foliar application was used in the chickpea grain filling phase.

On the other hand, environmental temperature can controls the opening mechanism of the plant stomata. In this study, rainfed chickpea may be affected by water scarcity and increased environmental temperature during reproductive growth phase that prevent the opening of stomata apertures and absorption of amino acid. In the similar research, Shehata et al. (2016) in two year study were found that amino acids foliar application significantly affected the total soluble solids percentage and fruits weight while concentration of nitrate in fruits was not affected significantly.

The biosynthesis of chlorophyll and proteins depends from ability of the absorption more nitrogen either from amino acids applied or via increasing nitrogen uptake from the soil (Shekari and Javanmardi, 2017). Probably, the applied amino acid, has prevented or reduced the function of atmospheric nitrogen fixation by symbiosis bacteria that coexist with the root of chickpea.

Also, amino acids may increase the nutritional value of chickpea seeds due to the accumulation of other nutrients that have not been studied in this experiment. Increasing the nutrients content were observed by amino acid in wheat grains such as: copper, sodium, calcium and molybdenum (Popko et al., 2018), iron in leaves and pods (Souri et al., 2018), the amount of nitrogen in transplanted plant (Shekari and Javanmardi, 2017).

In this experiment, the effect of nano-fertilizers on the protein content was also not significant. Mahajan et al. (2011) announced that accumulation and uptake of nanoparticles was dependent on the exposure concentration.

Yadav and Shukla (1983) declared that zinc in lower concentration could increase number of nodules and leghaemoglobin content of nodules, nitrogen fixation and finally yield, but in higher concentration nodulation and nitrogen fixation decreased.

Also, Janmohammadi et al. (2016a) stated that plant response to nanoparticles significantly depend on concentration and time of application as well as size, shape, and surface of the particles.

Finally, in this experiment protein yield, which resulted in protein percent in grain yield, was increased significantly compared with a control by amino acid and nano-fertilizers.

CONCLUSIONS

In general, the results of this study showed that application of pluramin significantly increased the number of secondary branches, number of pods, number of seeds per pod, 100 seed weight, grain yield, biomass and protein yield. Therefore, the application of pluramin was evaluated as favorably. According to the results, the main reason for increasing the grain yield of chickpea in this experiment was increasing the number of secondary branches and all yield components under the influence of pluramin. In this experiment, the most of studied traits were increased by iron and zinc nano-fertilizers compared with control.

However, iron+zinc nano-fertilizers were superior to other treatments due to a significant increase in 100 seed weight, which ultimately led to an increase in grain yield. Finally, the results of interaction effects on studied traits including grain yield as the most important trait showed that maximum grain yield was obtained in the treatment of 250 grams of pluramin and iron+zinc nano-fertilizers.

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