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## STATISTICAL ASSESSMENT OF THE IMPACT OF NANO-CHELATED ELEMENTS AND SULFUR ON CHICKPEA PRODUCTION UNDER SUPPLEMENTAL IRRIGATION

#### SUMMARY

Chickpea is an important source of plant protein source and has a major role at people nutrition in semi-arid regions. Soils of these regions have high pH and low organic matter, which reduce the availability of most micronutrients. In order to investigate the effects of application of sulfur (0, 15, 30 kg ha<sup>-1</sup>) and three nano-chelated micronutrients (nano-Zn, nano-Fe and nano-Mn) on yield and some morphological traits of chickpea, a field experiment was conducted. Day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), number of seeds per plant (NSP), seed yield (SY), straw yield (ST), biological yield (BY), harvest index (HI), and 1000 seed weight (TSW) were measured. Results showed that the first two principal components (PC1 and PC2) were used to create a two-dimensional treatment by trait (TT) biplot that accounted percentages of 53% and 26% respectively of total variation. The vertex treatments in polygon of TT biplot were S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano1, and S3-Nano1 which S3-Nano1 treatment combination indicated high performance in DM, FPH, PBP, SBP, NPP, NSP, SY, ST, BY and TSW. According to ideal treatment biplot, the S3-Nano1 (30 kg ha<sup>-1</sup> sulfur plus nano-chelated zinc) might be used in selecting superior traits and it can be considered as the candidate treatment for chickpea production. Treatment combinations which are suitable for obtaining of high seed yield performance were identified in the vector-view biplot and showed S3-Nano1 as the best treatment suitable for obtaining of high seed yield. In conclusion, application of nano-fertilizer could increase crop yield and improve the fertilizer efficiency.

Keywords: Nano-manganese, Nano-iron, Nano-zinc, micronutrients.

## **INTRODUCTION**

Chickpea is a food legume, which ranks third after beans and field peas among the world's pulse crops and is grown in a wide range of environments in many countries around the world because it is a rich source of quality protein for a majority of the population (FAO, 2014). It is cultivated on an area of about 12

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million hectares with an annual production of 9.5 million tones and an average productivity of 880 kg ha1 while in Iran, it is cultivated on 550 thousand hectares annually producing 300 thousand tons with an average productivity of 540 kg ha-1 (FAO, 2014). The country ranks sixth after major chickpea producing countries include India, Australia, Pakistan, Turkey and Myanmar. One of the main reasons for chickpea low productivity in Iran is cultural management such as application of fertilizers (Johnson et al. 2005). Micronutrients are needed in very small quantities for optimum crop growth and play a vital role in human health while the lack of trace elements or imbalance among them may result in growth suppression or even inhibition of crop productivity of crops but the nutrient use efficiencies of conventional fertilizers is relatively low and relatively few investigations have analyzed the application of the new micronutrient fertilizers on chickpea and agronomic managements for improving crop productivity (Subramanian et al. 2015).

Among different micronutrients, the deficiencies of zinc (Zn), iron (Fe) and manganese (Mn) are the most important yield-limiting factors in semi-arid regions because the availability of most of micronutrients is greatly depends on soil pH because high pH cause to unavailability of trace elements such as iron, manganese, zinc and sulfur application can be suitable option for solution of this problem (Plaster, 2013). Some studies showed that the soils of rainfed areas in arid and semi-arid environments are deficient in sulfur (Khalid et al. 2011; Islam, 2012). Also, information regarding effects of sulfur on micronutrients especially Zn, Fe and Mn availability and uptake by crop plants is very scarce. Zinc deficiency is common throughout the world and lack of Zn can limit the growth and productivity of a wide range of crops (Harris et al. 2008). Fertilizers containing Zn are commonly added to soils where necessary and it can also be effective to use foliar sprays. Mn deficiency is common in some soils and chickpea is the most sensitive on Mn deficiency and chloroplast ranks first among the organelles to Mn deficiency, which this deficiency symptoms was inversely related to root length while it is associated to the 100-grain weight (Bozoglu et al. 2008). The role of Fe as an essential nutrient and its function in metabolism have been investigated in detail by some investigations (Marschner, 1995; Fox et al. 1998), however, it is abundant in most soils especially under alkaline or calcareous conditions. Therefore, Zn, Fe and Mn micronutrients and sulfur are essential elements for crop growth because it can affect the crop development and plays some critical rules in crops and given the importance of chickpea, it seems that one way of improving the its low productivity can be application of new nano-fertilizers micronutrients.

Nanoparticles are one potential output that could be a major innovation for agriculture (Derosa et al. 2010). Nano-fertilizers could be more soluble or more reactive than bulk fertilizers and they can exactly release their active ingredients in responding to environmental triggers (Mastronardi et al. 2015). Nano-sized nutrients could be perhaps predicted to have a considerable effect on fertilizer

efficiency and crop productivity. However, beneficial aspects of nano-particles have now been explored by researchers in different fields (Nietzold and Lisdat, 2012). The importance of nano-fertilizers for improving saffron yield was reported also by Amirnia et al. (2014), who found that application of iron nano-particles enhanced chlorophyll, carbohydrate, essential oil, fresh weight and dry weight, and foliar spray treatment was found to be more effective than soil addition of nano-fertilizer. According to Soliman et al. (2015), iron nano-particles applied in the form of spray on soybean increased dry weight. The present field survey was conducted to investigate the effect of sulfur fertilizer and three nano-form of zinc, iron and manganese on yield and some other traits of chickpea crop in rainfed condition of semi-arid area under supplemental irrigation.

### MATERIAL AND METHODS

A field experiment was conducted using cultivar Kakaie of chickpea at Takab district (47°70' E; 36°23' N), Northwest of Iran with an average annual rainfall of 340 mm and mean annual temperature of 12.3 °C. The trial was performed in a split-plot experiment according to randomized complete block design with three replication (plot size of  $2 \times 2 \text{ m}^2$ ) keeping sulfur in main plots and nano-chelated micronutrient in sub plots. The soil texture was sandy loam, with 7.8 pH, with0.44% organic carbon, 0.044% N, 4.34 mg. kg<sup>-1</sup> P, and 227 mg. kg<sup>-1</sup> K and the recommended NPK fertilizer was 30 kg N and 75 kg  $P_2O_5$  ha<sup>-1</sup>. Sulfur fertilizer was applied in three levels (S1: no application, S2:15 Kg ha<sup>-1</sup>, S3:30 Kg ha<sup>-1</sup>) were mixed with top soil and nano-chelated micronutrients were including N1: nano-chelated zinc, N2: nano-chelated iron, and N3: nano-chelated manganese. Nano-chelated fertilizers were applied at rate of 1 kg ha<sup>-1</sup> through fertigation 30 and 60 days after sowing date. Nano chelate fertilizers were obtained from the Sepeher Parmis Company, Iran, which contained zinc oxide, ferric oxide and manganese (II) oxide nanoparticles with morphological properties which were characterized by scanning electron microscope (Figure 1).



Figure 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles of zinc oxide utilized for nano-chelated fertilizer.

Chickpea was sown manually in the third week of April in 10 rows, at 20 cm row-to-row spacing and 8 cm plant-to-plant spacing. Two supplemental irrigations applied during flowering and grain filling stages. The amount of irrigation water was calculated to restore water content in the root zone to field capacity. Weeds were controlled by frequent hand weeding and. Traits day to maturity (DM) was recorded for each experimental plot. Plants were harvested by hand at June and some agronomic traits including first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), and number of seeds per plant (NSP) were recorded on 10 randomly selected plants in each plot. Seed yield (SY), straw yield (ST) and biological yield (BY) were determined by harvesting the middle three rows of each plot after avoiding border effects and harvest index (HI) of each plot was calculated according to the ratio of seed yield to biological yield. The 1000 seed weight (TSW) was measured after harvesting and drying from three random sample of each plot. The two-way layout of treatment × trait (TT) biplot model is used according to (Yan and Rajcan, 2002) via GGEbiplot software (Yan, 2001).

### **RESULTS AND DISCUSSION**

The analysis of treatment  $\times$  trait interaction through biplot analysis indicated that the first and second principal components (PC1 and PC2) analysis together could explain 79% of the total variation. The polygon-view biplot (Figure 2), indicated the nine treatment combinations (3 sulfur levels  $\times$  3 nanochelate fertilizer levels) fell into three sections of five possible sections and the measured traits could be grouped into three groups, suggesting that number of empty pod per plant (EPP) and harvest index (HI) could be identified as different traits from the other remained traits. The vertex treatment combinations were S3-Nano1, S1-Nano2, S1-Nano3, S1-Nano1, and S2-Nano1 which S1-Nano3 treatment had better performance in EPP; S1-Nano2 treatment had better performance in HI. Also, S3-Nano1 treatment indicated high performance in day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of seeds per plant (NSP), seed yield (SY), biological yield (BY), and 1000 seed weight (TSW) traits (Figure 2). Chickpea responded positively to the Zn application (Roy et al. 2006) and Brennan et al. (2001) reported the relative response of chickpea to Zn application is greater than that of other crops. As with other pluses, nano-Zn application resulted in more vegetative growth (Singh et al. 1992), leading to higher dry matter production and greater seed yield. Also, Zn application increased significantly chickpea growth and development (Khan et al. 2000) and thus at maturity plants fertilized with nano-Zn had a greater total dry weight.



Figure 2. Polygon view of TT biplot showing which fertilizer treatment combination had the highest values for which traits

The vector-view biplot indicates the relationships among traits by the angles between the vectors of traits, which are the lines that connect each trait point with the origin point of the biplot and the cosine of the angle between vectors approximately represents the correlation between two traits, and an acute angle indicates a positive correlation, otherwise a negative correlation between the two traits (Yan and Tinker, 2006). The NPP, TSW, BY, SBP, DM and PBP traits are positively correlated because of the acute angles among their vectors (Figure 3). Also, ST with FPH, and NSP, SY and HI were positively correlated due to their acute angles. A near zero correlation between ST and FPH with EPP, between NSP, SY and HI with HI, between EPP, and between NSP, SY and HI with NPP, TSW, BY, SBP, DM and PBP as indicated by the near perpendicular vectors (Figure 3). Also, a negative association between NSP, SY and HI with ST and FPH and between EPP with NPP, TSW, BY, SBP, DM and PBP as indicated by the large obtuse angles (Figure 3). These results are in good accordance with those reported by Noor et al. (2003). According to Yucel et al. (2006) the chickpea yield showed positive correlation with first pod height, number of secondary branch, number of total full pods, number of full pods per plant, and number of seeds per plant. The main purpose of farmers is to achieve high yield in chickpea which its components are multi-genic traits, and are influenced by the environmental factors such as fertilizer application. The TT biplot analysis of chickpea's dataset shows visual interrelationships among the traits, which provides more information in comparison to correlation coefficients that only describe the relationships between two traits (Janmohammadi et al. 2015). Most of the above results can be grasped from the simple correlation coefficients (Table 1), but some others are not consistent with them and such discrepancies are observed because the TT biplot explained lower than 100% (in present study, 79%) of the variation



Figure 3. Vector view of TT biplot showing the interrelationship among measured traits under different fertilizer treatment combinations.

The ideal trait is a tester that is most discriminating and representative among all traits (Yan and Tinker, 2006) and the center of concentric circles on the average tester coordinate indicates the ideal trait (Figure 4). The distance from the ideal trait to the biplot origin is equal to the longest vector of all traits and so TSW might be used in selecting superior treatments, which it could be useful in achieving high performance via application of different fertilizer treatments. In other word, TSW is good indicator for discriminant among different sulfur and nano-chelated fertilizer treatments. The ranking of the other traits based on ideal trait were: NPP > BY > SBP > DM > PBP. The concept of ideal treatment is the entry that is most favorable treatment among all treatments (Yan and Tinker, 2006) and it has been shown that the distance between one

treatment and the ideal treatment is a more repeatable parameter to evaluate the treatments performance.



Figure 4. Ideal entry view of TT biplot, showing the relationships of different fertilizer treatment combinations with ideal entry.

In a TT biplot, the center of the concentric circles on the average tester coordinate indicates the ideal treatment (Figure 5), which is equal to the length of treatment vector with the highest performance and so the distance between the ideal treatment and the biplot origin is equal to the longest vector among all treatments. Thus, the S3-Nano1 (30 kg ha-1 sulfur plus nano-chelated zinc) might be used in selecting superior traits and it can be considered as the candidate treatment. The performance of measured traits of chickpea under application of S2-Nano1 (15 kg ha-1 sulfur plus nano-chelated zinc), S3-Nano2 (30 kg ha-1 sulfur plus nano-chelated iron) and S3-Nano3 (30 kg ha-1 sulfur plus nano-chelated manganese) treatment combinations were observed above average while the other treatments (S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano2 and S2-Nano3) were below average (Figure 5).

Suitable treatment combinations for obtaining of high seed yield (SY) of chickpea could be identified in the biplot of Figure 6 which is a vector-view function of TT biplot model and shows treatments that have close association with a target trait among other traits. Based on this type of biplot, S3-Nano1 (30

Table 1. Pearson's simple correlation coefficients among chickpea traits.											
	FPH	PBP	SBP	DM	NPP	EPP	NSP	TSW	SY	BY	ST
FPH											
PBP	0.17										
SBP	0.40	0.82									
DM	0.34	0.79	0.86								
NPP	0.24	0.32	0.49	0.74							
EPP	-0.18	-0.29	-0.66	-0.63	-0.82						
NSP	-0.17	0.09	0.29	0.17	0.46	-0.66					
TSW	0.11	0.64	0.82	0.84	0.65	-0.68	0.48				
SY	-0.10	0.23	0.45	0.38	0.52	-0.68	0.93	0.66			
BY	0.19	0.57	0.86	0.83	0.67	-0.78	0.40	0.95	0.55		
ST	0.31	0.51	0.68	0.70	0.41	-0.41	-0.23	0.63	-0.10	0.77	
HI	-0.24	-0.08	0.02	-0.04	0.22	-0.35	0.87	0.22	0.87	0.06	-0.59

kg ha-1 sulfur plus nano-chelated zinc) treatment combination was identified as optimal fertilizer treatment suitable for obtaining high seed yield.

Critical values of correlation P<0.05 and P<0.01 (degrees of freedom = 7) are 0.67 and 0.75, respectively.



Figure 5. Ideal tester view of TT biplot, showing the relationships of different traits with ideal tester.

Traits were: day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), number of seeds per plant (NSP), seed yield (SY), straw yield (ST), biological yield (BY), harvest index (HI) and 1000 seed weight (TSW).

Thus, application this treatment is expected to lead to improved seed yield under supplemental irrigation conditions in semiarid environment. The ranking of the treatment combinations based on high performance of SY were: S3-Nano2 > S2-Nano3 > S1-Nano2. The best treatments combinations for obtaining of high TSW of chickpea could be determined from the vector-view biplot of Figures 3 and 6, and it indicates treatments that have close association with a target trait among other traits. According to biplot of Figures 3 and 6, S3-Nano1 (30 kg ha-1 sulfur plus nano-chelated zinc) treatment combination was identified as optimal fertilizer treatment suitable for obtaining high TSW and the ranking of the other treatment combinations based on high performance of TSW were: S3-Nano2 > S2-Nano1 > S2-Nano2 > S3-Nano3. This suggests that using nano-sized micronutrient fertilizer will not only result in the development of high seed yield but also cause to achieving desirable TSW trait as well as the other yield components that enhance wide use of such treatment.

Bala et al. (2014) have observed beneficial role of nano-fertilizer application in chickpea crop growth due to increase in activity of growth hormone gibberellins. Liu et al. (2010) found that nano-particles were safe for seed germination and growth of wheat seedlings and also conclude that use of nano-sized fertilizers is useful in crop production. Kharol et al. (2014) showed that application of sulfur and zinc increased the yield performance of chickpea and application of sulfur recorded fifty percent higher in seed yield over control treatment. Due to widespread deficiency of sulfur and Zn in the semiarid regions because of poor (Srinivasarao et al. 2006) and depletion under continuous cropping without application of these nutrients (Rego et al. 2007), their application caused to significant increase in most traits of chickpea. Our results clearly demonstrated significant seed yield responses of different rainfed crops due to application of Zn and sulfur. The deficiencies of Zn and sulfur nutrients assume critical importance for increasing and sustaining chickpea crop productivity of rainfed conditions.

It could be concluded that the TT biplot analysis is excellent graphical tool for visual data analysis and similar reports demonstrated that its efficiency for visualizing treatment-by-trait data and revealing the interrelationships among traits (Peterson et al. 2005). The TT biplot provided a proper tool for visual comparison among treatment combinations on the basis of multiple traits and it effectively revealed the interrelationships among the treatment combinations (sulfur levels in nano-fertilizers). Yan and Kang (2003) suggested that, if there are no clear cut tester by entry pattern, a TT biplot based on values across all treatments should be suffice and if there are clear groups of treatments.



Figure 6. Vector view of TT biplot, showing the relationships of different fertilizer treatment combustions with target trait (TSW, thousand seed weight).

#### CONCLUSIONS

This research indicated that nano-chelated Zn and sulfur application increase seed yield, primarily due to an increase in the number of seeds per plant, secondary due to an increase in the harvest index, tertiary due to an increase in the number of pod per plant. High levels of sulfur fertilizer (30 kg ha-1) and nano-Zn can cause a significant increase in seed yield and 1000 seed weight. Nano-Zn and sulfur application can increase the yield of chickpea cultivated in semiarid soils with supplemental irrigation.

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