

Graphic Analysis of Nano-Sized Fertilizers Treatment × Trait Interaction in Chickpea (*Cicer arietinum* L.)

N. Sabaghnia¹, S. Yousefzadeh^{2,*} and M. Janmohammadi¹

¹ Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

² Department of Agriculture, Payame Noor University, P.O.Box 19395-3697, Tehran, Iran

* Corresponding author: Email: s_yousefzadeh@pnu.ac.ir

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ABSTRACT

The aim was to explore the application effect of different nano-sized fertilizers (zinc, iron and manganese) and sulfur fertilizer on chickpea. The sulfur fertilizer had three levels (S1 : no application, S2 : 15 Kg ha⁻¹, S3 : 30 Kg ha⁻¹) and the nano-fertilizer was including nano-chelated zinc (Nano1), nano-chelated iron (Nano2), and nano-chelated manganese (Nano3). According to results the first two principal components (PC1 and PC2) were used to create a two-dimensional treatment by trait (TT) biplot that accounted percentages of 61% and 19% respectively of sums of squares of the TT interaction. The vertex treatments in polygon of TT biplot were S3-Nano1, S3-Nano3, S1-Nano1, S1-Nano2, and S1-Nano3 which S3-Nano1 treatment indicated high performance in days to 50% flowering, plant height, primary branch per plants, secondary branch per plant, first pod height, 1000 seed weight and seed yield. The identification of ideal treatment, the treatment that is most favorable treatment among all ones, showed that 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. Treatments suitable for obtaining of high seed yield were identified in the vector-view function of TT biplot and displayed S3-Nano1 (30 kg ha⁻¹ sulfur plus nano-chelated zinc) as the best treatment suitable for obtaining of high seed yield.

Keywords: Nano-fertilizer, nano-iron, nano-manganese, nano-zinc

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) as none of the cool season legumes in the world and its seeds are used for human nutrition, as they contain a high level of proteins, carbohydrates, and dietary fibers (Jukanti *et al.*, 2012). It improves soil fertility by meeting most of its nitrogen demand from symbiotic nitrogen fixation process. It is cultivated on 550 thousand ha annually producing 300 thousand tons of grain with an average productivity of 536 kg ha⁻¹ in Iran (FAO, 2013). The country ranks sixth and contributes about 3% of global production and the other major chickpea producing countries

include Australia, Pakistan, Turkey and Myanmar (FAO, 2013). Of the world production, 84.5% is produced in Asia, 4.1% in Africa, 4.5% in North and Central America (mainly Canada and Mexico), 6.2% in Oceania (mainly Australia), and 0.7% in Europe. Despite of a large area under cultivation, there exists a wide gap between maximum world potential (3400 kg ha⁻¹ in Republic of Moldova) and Iran's average productivity. One of the main reasons for low productivity is cultural management of commercially grown chickpea cultivars and application of fertilizers is the major limiting chickpea productivity worldwide (Johnson *et al.*, 2005).

Some surveys were performed for assessing the sulfur (S) status of soil which showed that the soils of rainfed areas in arid and semi-arid environments are deficient in sulfur (Khalid *et al.*, 2011; Islam, 2012). Sulfur fertilizer is known to enhance crop yield and uptake of macronutrients especially nitrogen and its application to alkaline soils has been reported to reduce the pH of soil (Taalab *et al.*, 2008; Islam *et al.*, 2012). Also, information regarding effects of sulfur on micronutrients especially zinc (Zn), iron (Fe) and manganese (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. Zn deficiency in soils was widespread and it has estimated that about 70% of the cultivated area of arid and semi-arid areas has Zn deficiency and that Zn deficiency which is the third most serious crop nutrition problem in the country after nitrogen and phosphorus deficiency (Harris *et al.*, 2008). Fertilizers containing Zn are commonly added to soils where necessary and it can also be effective to use foliar sprays.

Manganese (Mn) deficiency is common in some soils and levels above 20–25 ppm are adequate for crop growth. Legume plants such as 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. The physicochemical properties of Fe dictate the formation of highly insoluble Fe oxides and hydroxides, making Fe limiting for crop growth and its solubility in mineral soils is too low to fulfil the crop demand (Mahmoudi *et al.*, 2007).

The importance of nano-fertilizers was reported by Amirnia *et al.* (2014), who found that application of iron nano-particles enhanced chlorophyll, carbohydrate, essential oil, fresh weight and dry weight of saffron. According to Soliman

et al. (2015), iron nano-particles applied at the concentration of 0.75 g (dm³)⁻¹ in the form of spray on soybean increased dry weight, and application of 0.5 g (dm³)⁻¹ concentration resulted in the highest yield, showing 48% increase in comparison with the control. In this way, the present field survey was conducted to investigate the effect of sulfur fertilizer and three nano-form of zinc, iron and manganese on seed yield and other traits of chickpea crop.

MATERIALS AND METHODS

Field experiments were conducted using chickpea cultivar of Kakaiefarmer field, Takab district (The northwest of Iran) during crop growing season of 2014–2015 which is located at an altitude of 1765 meter above sea level and is representative of upland semi-arid region. The climate is identified as a cold semi-arid and with an average annual rainfall of 340 mm and the mean annual temperature was 12.3°C. The precipitation was 120.5 mm and the relative humidity ranges between 33–63% during the cropping season. The soil texture of the experimental site in the 0–40 cm layer was sandy loam, with 7.8 pH, EC of 0.78 dS m⁻¹, and soil analysis indicated 0.044% total nitrogen, 0.44% organic carbon, 4.34 mg kg⁻¹ available P, 227 mg kg⁻¹ available K.

The recommended fertilizers (30 kg nitrogen and 75 phosphorus kg ha⁻¹) were applied in the form of urea and triple superphosphate at the time of seed bed preparation. The trial was laid out as split plot (2 × 2 m plot) basis on Randomized Complete Block Design (RCBD) keeping sulfur fertilizer in main plots and three nano-chelated micronutrient fertilizers (nano-zinc, nano-iron and nano-manganese) in subplots. The sulfur fertilizer had three levels (S1 : no application, S2 : 15 kg ha⁻¹, S3 : 30 kg ha⁻¹) which were mixed with top soil and the nano-chelated micronutrient was including Nano1 : nano-chelated Zinc, Nano2 : nano-chelated Iron, and Nano3 : nano-chelated manganese. The sulfur fertilizer was spread over the soil surface and incorporated into the top 10 cm of soil while foliar application of nano-chelated fertilizers was applied at rate of 1 kg ha⁻¹ at 30 and 60 days after sowing date. Nano-chelate fertilizers

were obtained from the Sepeher Parmis Company (one of the most producers of nano-fertilizers), Iran, which contained zinc oxide, ferric oxide and

manganese (II) oxide nanoparticles and they were characterized morphologically by scanning electron microscope (Figure 1).

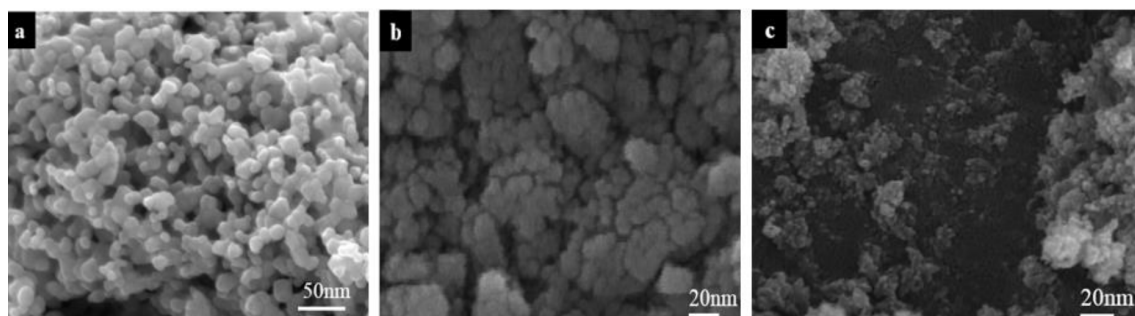


Figure 1 Scanning Electron Microscope (SEM) image of synthesized nanoparticles of zinc oxide (a), ferric oxide (b) and manganese (II) oxide (c) and utilized for nano-chelated fertilizers

Seeds was sown manually in the third week of April into 10 rows, at 20 cm row-to-row spacing and 8 cm plant-to-plant spacing. Weeds were controlled by frequent hand weeding and irrigation was performed after planting and four times of irrigation applied during growth period. Phonological growth phases including days to 50% flowering (DF) and day to maturity (DM) was recorded for each experimental plot. Plants were harvested by hand at June and some agronomic traits including plant height (PH), 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) was 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 treatment \times trait (TT) biplot model (Yan and Rajcan, 2002) is used based this formula.

$$\frac{\alpha_{ij} - \beta_j}{\sigma_j} = \sum_{n=1}^2 \lambda_n \xi_{in} \eta_{jn} + \varepsilon_{ij} = \sum_{n=1}^2 \xi_{in}^* \eta_{jn}^* + \varepsilon_{ij}$$

where α_{ij} is the mean value of treatment i for trait j , β_j is the mean value of all treatments in trait j , σ_j is the standard deviation of trait j among the treatment means, λ_n is the singular value for principal component n (PCn), ξ_{in} and η_{jn} are scores for treatment i and trait j on PCn, respectively, and ε_{ij} is the residual associated with treatment i in trait j . Visual analysis of dataset via TT biplot was performed using GGEbiplot software (Yan, 2001). Yan *et al.* (2000) have developed a genotype main effect plus genotype by environment (GGE) biplot methodology for the graphical analysis of multi-environment trial data. This method can be used for all types of two-way dataset such as treatments with multiple traits. Sabaghnia *et al.* (2011) have used a treatment by trait (TT) biplot, which is an application of the GGE biplot to study the treatment by trait data for yield analysis of rapeseed under water-stress conditions.

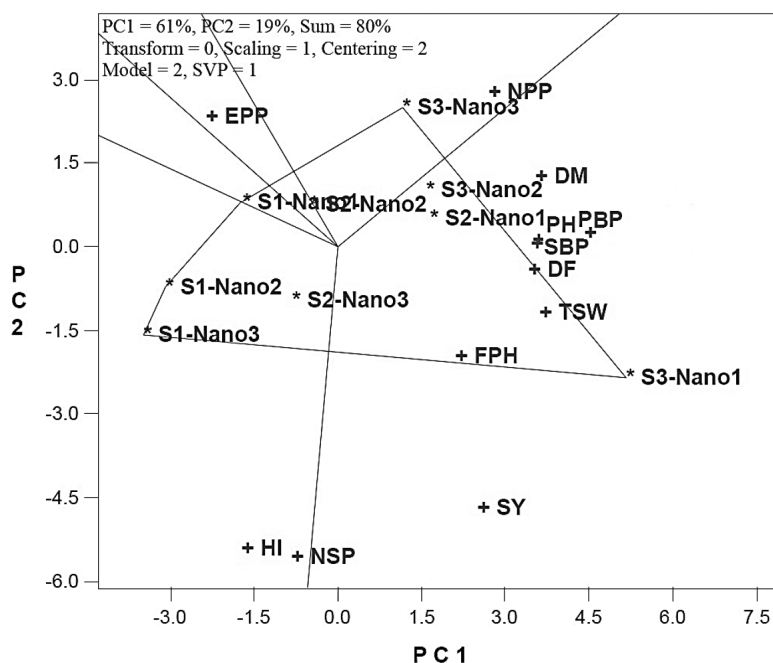


Figure 2 Polygon view of treatment by trait (TT) biplot showing which fertilizer treatment combination had the highest values for which traits. For traits abbreviations, refer to the text

RESULTS AND DISCUSSION

The obtained results showed that the partitioning of TT interaction through biplot analysis showed that the first and second principal components (PC1 and PC2) together could explain 80% of the total variation. From the polygon view of biplot analysis (Figure 2), the treatment combinations (sulfur by nano-chelate) fell into five sections and the traits could be grouped into four sections, suggesting that NPP, EPP, NSP and HI could be identified as different traits from the other remained traits. The vertex treatments were S3-Nano1, S3-Nano3, S1-Nano1, S1-Nano2, and S1-Nano3 which S3-Nano3 treatment had better performance in NPP; S1-Nano1 treatment had better performance in EPP; S1-Nano3 treatment had better performance

in NSP and HI. Also, S3-Nano1 treatment indicated high performance in DM, PH, PBP, SBP, TSW, FPH and SY (Figure 2). Chickpea responded to the Zn application even though nano-Zn availability (Roy *et al.*, 2006); however, Brennan *et al.* (2001) reported that the relative response of chickpea to Zn application is greater than that of other crops. As with other leguminous crops, nano-Zn application resulted in more vegetative growth (Singh *et al.*, 1992), leading to higher dry matter production and greater seed yield. Zn application increased chickpea growth (Khan *et al.*, 2000) and thus at maturity plants fertilized with nano-Zn had a greater total dry weight. A relationship between Zn application and seed yield of chickpea was reported (Brennan *et al.*, 2001).

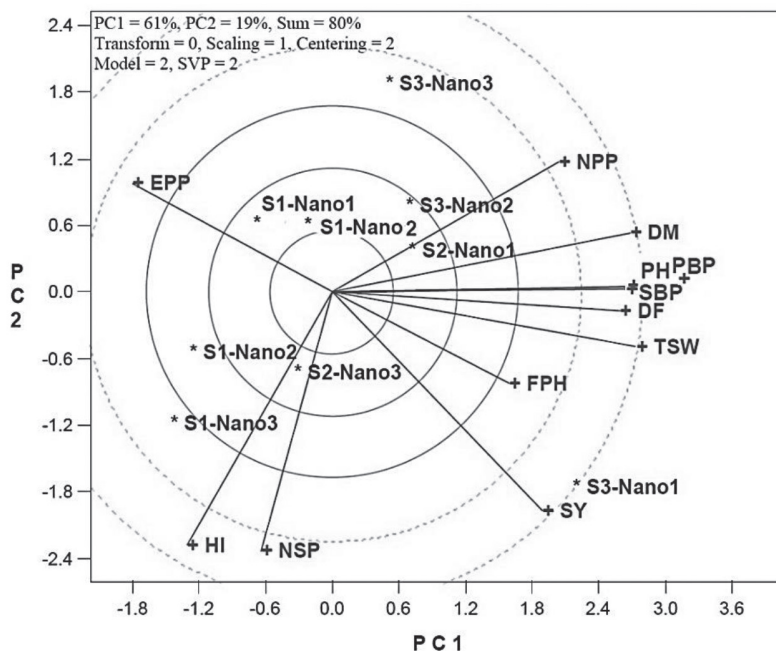


Figure 3 Vector view of treatment by trait (TT) biplot showing the interrelationship among measured traits under different fertilizer treatment combinations. For traits abbreviations, refer to the text

In the vector-view biplot Figure, the relationships among traits are determined by the angles between the trait vectors, which are the lines that connect each trait point with the origin point of the biplot (Figure 3). The DM, PBP, PH, SBP, DF and TWS traits are positively correlated because of the acute angles among their vectors (Figure 3). Also, HI with NSP and FPH and SY were positively correlated due to their acute angles. A near zero correlation between NPP with EPP, between FPH with HI, between NSP and HI with EPP, and between NSP and HI with DM, PBP, PH, SBP, DF and TWS as indicated by the near perpendicular vectors (Figure 3). Also, a negative association between NPP with HI and NSP and between EPP with SY as indicated by the large obtuse angles (Figure 3). These results were in accordance with

those reported by Guler *et al.* (2001) and Noor *et al.* (2003). Uddin *et al.* (1990) indicated that an increase in plant height leads to a decrease in number of secondary branch. According to Yucel *et al.* (2006) the chickpea seed yield exhibited a significant positive correlation with plant height, 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 producers is to achieve an increase in chickpea yield which its components are multigenic traits, which are strongly influenced by the environment such as fertilizer application and other factors both known and yet to be identified. To this end, emphasis should be given to the development of chickpea lines with higher first pod height. Application of TT biplot to this investigation on chickpea 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). Although most of the above predictions can be verified from the Pearson's correlation coefficients (Table 1), but some others are not consistent with the original coefficients of correlation because

such discrepancies are seen because the TT biplot method explained lower than 100% (in present study, 80%) of the total variation. Although, all above conclusions have some errors but TT biplot shows predictions on the general pattern of the whole dataset, the predictions are probably more reliable than the individual observations.

Table 1 Pearson's simple correlation coefficients among chickpea

Traits	PH	FPH	DF	CW	PBP	SBP	VGP	DM	NPP	EPP	NSP	TSW	SY	BY	ST
FPH	0.45 ^{ns}														
DF	0.92 ^{**}	0.48 ^{ns}													
CW	0.89 ^{**}	0.53 ^{ns}	0.91 ^{**}												
PBP	0.83 ^{**}	0.39 ^{ns}	0.76 [*]	0.64 ^{ns}											
SBP	0.92 ^{**}	0.55 ^{ns}	0.93 ^{**}	0.90 ^{**}	0.89 ^{**}										
VGP	0.51 ^{ns}	0.31 ^{ns}	0.40 ^{ns}	0.48 ^{ns}	0.72 [*]	0.65 ^{ns}									
DM	0.85 ^{**}	0.47 ^{ns}	0.84 ^{**}	0.83 ^{**}	0.88 ^{**}	0.95 ^{**}	0.83 ^{**}								
NPP	0.63 ^{ns}	0.20 ^{ns}	0.67 [*]	0.59 ^{ns}	0.70 ^{**}	0.73 [*]	0.80 ^{**}	0.88 ^{**}							
EPP	-0.53 ^{ns}	-0.47 ^{ns}	-0.42 ^{ns}	-0.41 ^{ns}	-0.69 [*]	-0.57 ^{ns}	-0.35 ^{ns}	-0.46 ^{ns}	-0.04 ^{ns}						
NSP	-0.23 ^{ns}	-0.04 ^{ns}	-0.17 ^{ns}	-0.26 ^{ns}	-0.14	-0.19 ^{ns}	-0.32 ^{ns}	-0.30 ^{ns}	-0.33 ^{ns}	0.01 ^{ns}					
TSW	0.88 ^{**}	0.63 ^{ns}	0.88 ^{**}	0.79 ^{**}	0.91 ^{**}	0.93 ^{**}	0.55 ^{ns}	0.85 ^{**}	0.56 ^{ns}	-0.77 [*]	-0.15 ^{ns}				
SY	0.64 ^{ns}	0.43 ^{ns}	0.71 [*]	0.57 ^{ns}	0.61 ^{ns}	0.65 ^{ns}	0.15 ^{ns}	0.52 ^{ns}	0.25 ^{ns}	-0.60 ^{ns}	0.45 ^{ns}	0.76 [*]			
BY	0.91 ^{**}	0.43 ^{ns}	0.89 ^{**}	0.85 ^{**}	0.89 ^{**}	0.95 ^{**}	0.56 ^{ns}	0.87 ^{**}	0.59 ^{ns}	-0.70 [*]	-0.03 ^{ns}	0.93 ^{**}	0.79 [*]		
ST	0.92 ^{**}	0.38 ^{ns}	0.86 ^{**}	0.87 ^{**}	0.90 ^{**}	0.96 ^{**}	0.66 ^{ns}	0.91 ^{**}	0.65 ^{ns}	-0.66 ^{ns}	-0.21 ^{ns}	0.90 ^{**}	0.63 ^{ns}	0.97 ^{**}	
HI	-0.45 ^{ns}	-0.02 ^{ns}	-0.30 ^{ns}	-0.46 ^{ns}	-0.47 ^{ns}	-0.49 ^{ns}	-0.64 ^{ns}	-0.56 ^{ns}	-0.55 ^{ns}	0.18 ^{ns}	0.73 [*]	-0.31 ^{ns}	0.29 ^{ns}	-0.35 ^{ns}	-0.56 ^{ns}

Note: ns = non-significant; * = significant with 95% confidence level and ** = significant with 99% confidence level

Abbreviations are: days to 50% flowering (DF), day to maturity (DM), plant height (PH), 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), harvest index (HI) and 1000 seed weight (TSW)

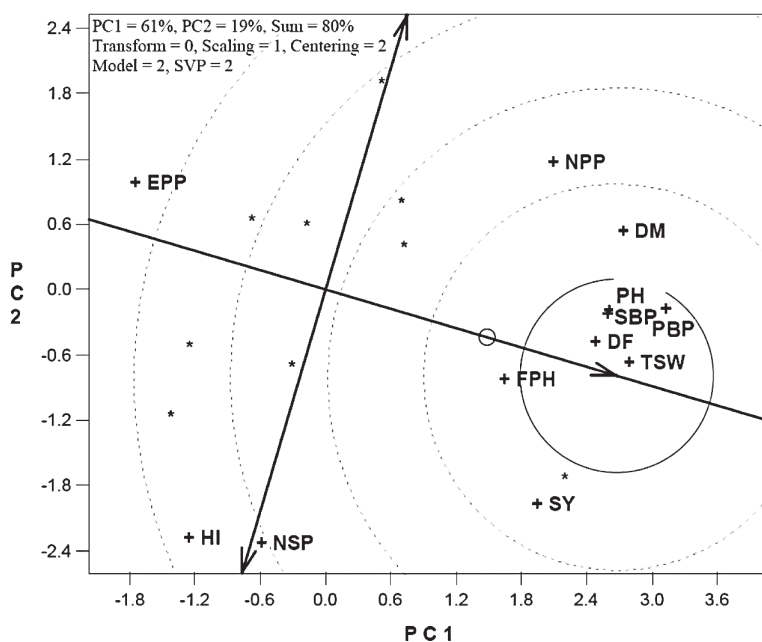


Figure 4 Ideal tester view of treatment by trait (TT) biplot, showing the relationships of different traits with ideal tester. For traits abbreviations, refer to the text

The concept of 'ideal trait' is defined as the trait that is most discriminating and also representative among all traits (Yan and Tinker, 2006). 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. Thus, PH, SBP, PBP, DF and TSW might be used in selecting superior treatments, which they could be useful in achieving high performance via application of different fertilizer treatments. In other word, the above mentioned traits were good indicators for discriminant among different sulfur and nano-chelated fertilizer treatments. The ranking of the other traits based on ideal trait were: FPH > SY = DM > NPP > NSP > HI > EPP. The concept of ideal treatment is the treatment that is most favorable treatment among all treatments (Yan and Tinker, 2006). It has been shown that the distance between one treatment and

the ideal treatment is a more repeatable parameter to evaluate the treatment performance. In a TT biplot Figure, 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. Therefore, 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. Also, the performance of various traits via application of S2-Nano1 (15 kg ha⁻¹ sulfur plus nano-chelated zinc) and S3-Nano2 (30 kg ha⁻¹ sulfur plus nano-chelated iron) treatment combinations were observed above average while the other treatments (S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano2, S2-Nano3 and S3-Nano3) were below average (Figure 5).

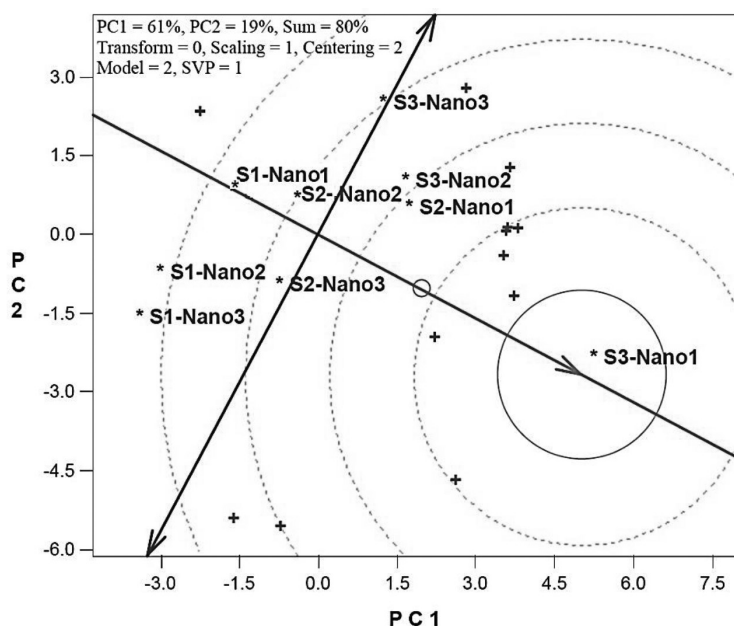


Figure 5 Ideal entry view of treatment by trait (TT) biplot, showing the relationships of different fertilizer treatment combinations with ideal entry. For traits abbreviations, refer to the text

Treatments suitable for obtaining of high seed yield (SY) could be seen in the biplot of Figure 6 which is a vector-view function of TT biplot model and displays treatments that have close association with a target trait among other traits. Based on this biplot, S3-Nano1 (30 kg ha⁻¹ sulfur plus nano-chelated zinc) treatment was identified as fertilizer treatment suitable for obtaining of high seed yield. Thus, selecting of this treatment is expected to lead to improved seed yield under optimal growing conditions. This suggests that using nano-sized micronutrient fertilizer will not only result in the development of high seed yield but also with other desirable agronomic traits as well as yield components that enhance wide use of such treatment. Bala *et al.* (2014) have observed beneficial role of

nano-fertilizer application in seed germination and plant growth regulation in chickpea due to increase in activity of growth hormone gibberellins. Also, Amirmia *et al.* (2014) have emphasized the positive effects of nano-fertilizers (iron, phosphorus and potassium) in the improvement saffron flowering traits. Liu *et al.* (2010) found that nano-particles were safe for wheat seed germination, emergence and growth of seedlings and also conclude that use of nano-sized fertilizers is useful in crop production as well as economic benefits. Kharol *et al.* (2014) indicated that application of increasing levels of sulfur and zinc increased the seed yield chickpea and application of sulfur (30 kg ha⁻¹) recorded fifty percent higher in seed yield and sulfur and zinc uptake over control.

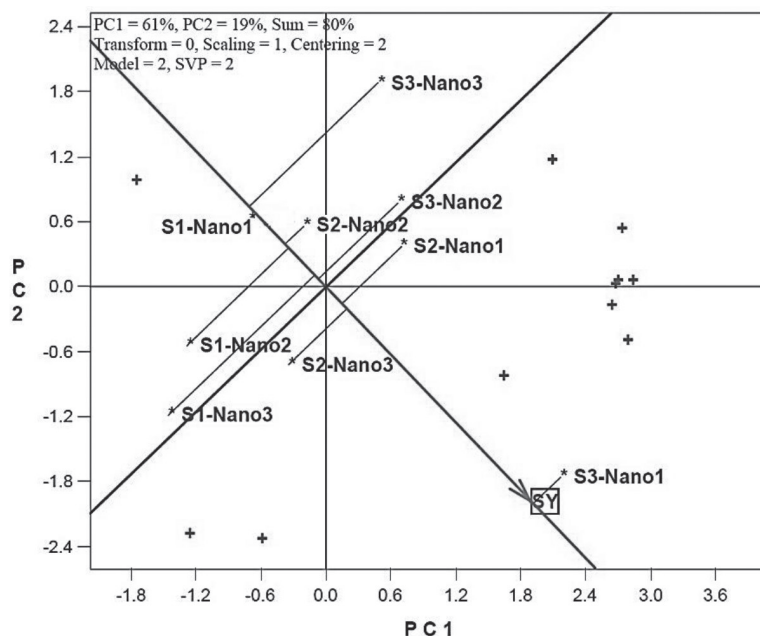


Figure 6 Vector view of treatment by trait (TT) biplot, showing the relationships of different fertilizer treatment combinations with target trait (SY, seed yield). For traits abbreviations, refer to the text

Due to widespread deficiency of sulfur and Zn in the semiarid regions because of poor organic carbon status of soils (Srinivasarao *et al.*, 2006) and depletion under continuous cropping without application of these plant nutrients (Rego *et al.*, 2007), application of these nutrients caused to significant increase in most traits of chickpea. Low levels of organic carbon in these soils were primarily due to high temperature, low rainfall and low or little organic matter additions (Rego *et al.*, 2003). Our results clearly demonstrated significant seed yield responses of different rainfed crops due to application of Zn and sulfur. The responses of crops to the application of Zn and sulfur varied across crops; the crop yields and nutrient uptake responses are clearly significant and are of similar

magnitude to those reported for field crops under irrigated agriculture (Fageria *et al.*, 2002; Rego *et al.*, 2007). Clearly, the deficiencies of Zn and sulfur assume critical importance for increasing and sustaining crop productivity of rainfed systems.

It could be concluded that the TT biplot model and its Figures are excellent tools for visual data analysis in agriculture. Similar reports demonstrated that the TT biplot was an excellent tool for visualizing treatment-by-trait data and revealing the interrelationships among traits (Peterson *et al.*, 2005; Fernandez-Aparicio *et al.*, 2009). In addition, it effectively reveals the interrelationships among the treatment combinations (sulfur levels in nano-fertilizers). Also, TT biplot provides a tool for visual comparison among treatment combinations on the

basis of multiple traits (Yan and Reid, 2008). Yan and Kang (2003) suggest 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, TT biplot should be constructed and studied for each cluster of treatments.

CONCLUSIONS

This study shows that nano-chelated Zn and sulfur applications increase seed yield, primarily due to an increase in the first pod height, secondary due to an increase in the 1000 seed weight, tertiary due to an increase in the days to 50% flowering and number of seeds per plant. High levels of sulfur fertilizer (30 kg ha⁻¹) and nano-Zn can cause

a significant increase in seed yield. Finally, the first pod height, 1000 seed weight, days to 50% flowering and number of seeds per plant are the most influential yield component, and the one that are most closely correlated with seed yield. Nano-Zn and sulfur application can increase the yield of chickpea cultivated in semiarid soils with normal irrigation.

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