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Analysis of the impact of nano-zinc, nano-iron, and nano-manganese fertilizers on chickpea under rain-fed conditions

ABSTRACT

Nanotechnology is an emerging field of science widely exploited in many scientific fields but its application in agriculture is rarely studied in the world. In the current study, application of nanotechnology in agricultural via the application of some micronutrient nano-fertilizers (nano-zinc, nano-iron, and nano-manganese) and different sulfur fertilizers have been investigated. Three levels of sulfur fertilizer (S1: no application, S2: 15 Kg ha^{-1}, S3: 30 Kg ha^{-1}) and three micronutrients nano-fertilizer (Nano1: nano-chelated zinc, Nano2: nano-chelated iron, and Nano3: nano-chelated manganese) were studied on some morphophysiological traits of chickpea. Results showed that the first two principal components of treatment × trait (TT) biplot accounted to 56% and 18% respectively of total variation. The vertex treatments in polygon biplot were S1-Nano2, S1-Nano3, S2-Nano1, S3-Nano1, and S3-Nano2 which S3-Nano1 treatment indicated high performance in day to maturity, plant height, first pod height, primary branch per plants, secondary branch per plant, number of pods per plant, number of seeds per plant and 1,000 seed weight. According to vector-view biplot, seed yield was positively associated with the number of pods per plant, harvest index and day to maturity. The ideal treatment identified the S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) that might be used in selecting superior traits and it can be considered as the candidate treatment. The ideal trait of biplot showed that seed yield had the highest discriminating ability and they were the most representative and as the final target trait of producers, it has the ability of discrimination among different treatments. The best fertilizer treatment for obtaining of high seed yield was identified in the vector-view function of TT biplot as S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc).

Keywords: nano-fertilizer, nanotechnology, yield components

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the main legume crop grown in the Mediterranean region, and Iran is the one of main chickpea-producers with 550,000 ha cultivated lands (about 13.5 million ha globally) and 295,000 t production (13.1 million t globally) in 2013 (FAO, 2013). It is a good source of plant protein, carbohydrates as well as minerals, vitamins and unsaturated fatty acids. Chickpea plays an important role in the human diet and cultivated worldwide. This crop is relatively tolerant to drought stress and is favorable options for semi-arid fields where the legume-cereal-fallow rotation is implemented. Despite its importance, few investigations have been conducted to study the micronutrients usage to chickpea due to their large impact of yield reduction. Despite its importance, few investigations have been conducted to study the role of micronutrients in chickpea due to their large impact of yield reduction. Although the chickpea is a rural crop, widespread deficiencies of micronutrients along with limited moisture supply are known as major environmental stresses causing yield loss (14). It is mainly cultivated as a rainfed crop and water shortage often affects the productivity as well as yield stability. Soils of semi-arid regions under rainfed cultivation are generally degraded with poor native fertility and micronutrients play an important role in increasing legume yield via their effects on the crop itself and the nitrogen-fixing process. Zn affect symbiotic N_2 fixation and it is known that its requirement is essential for fixation to occur, also, iron is required for several key enzymes of the nitrogenize complex as well as for the electron carrier ferredox in and for some hydrogenases while manganese plays an important roles in plant growth and development, also in detoxification of active oxygen species (5, 7). Micronutrient deficiencies in crops are becoming increasingly important globally due to intensive cultivation of high-yielding cultivars with heavy applications of nitrogen phosphorus and potassium fertilizers which cause to the occurrence of micronutrient deficiencies.

Iron (Fe) deficiency is often seen in high pH and calcareous soils in arid regions, similarly zinc and manganese (Mn) availability decrease with increasing of soil pH (8). significant increases in seed yield of chickpea by Zn application were demonstrated by Valenciano et al. (26), and in general, each tone of chickpea seed yield removes 38 g of Zn from the soil (1). Among micronutrients, Zn deficiency seems to be the most widespread (23) and it has considerable negative effect on chickpea productivity. Zn deficiency is very common among chickpea-growing regions of the world because chickpea is generally considered sensitive to Zn deficiency (14). In semi-arid region the main micronutrient that limits chickpea productivity is Zn and the critical Zn concentrations in soils vary from 0.48–2.50 mg kg^{-1} depending on soil type (1). Furthermore, Zn deficiency reduces water use efficiency (15) and also reduces nitrogen fixation (1), which contributes to a decrease in crop yield.

Likewise, Fe deficiency is a major constraint for many crops' production when grown in semi-arid areas and yield losses some pulse crops would likely occur due to Fe deficiency (33). According to Ghasemi-Fasaei et al. (6), application of Fe and Mn nutrients can increase shoot Fe uptake and its concentration in chickpea while the Mn availability in crops could be limited in semi-arid areas soils (9). In contrast, Fe application, however, may reduce yield performance caused by decreasing Mn and the negative effect of Fe application was attributed to the interference of Fe with Mn nutrition (22). The effect of Fe on Mn could be due to the increased yield performance that leads to dilution effect but accumulation of Fe to toxic levels cause to the reduction of Mn uptake (20).

Attempts are being made to synthesize nano-fertilizers in order to regulate the release of nutrients depending on the need of crops and in this context, some nano-fertilizers have been produced. Use of bulk fertilizers to increase the crop productivity is not a suitable option for long time due to environmental problems, while nano-fertilizers can increase the nutrient use efficiency through mechanisms such as targeted delivery, slow or controlled release and could precisely release their active ingredients in responding to environmental triggers and biological demands. Subramanian and Tarafdar (24) reported that nano-fertilizers can be used as a cementing material to regulate the release of nutrients from conventional fertilizers and this process increases the nutrient-use efficiencies and prevents environmental pollution. Also, nano-fertilizers have shown to increase the uptake and utilization of nutrients by crops (13). Bansiwal et al. developed a surface modified zeolite to slow release phosphate fertilizer particles which can be coated with nano-membranes that facilitate in slow and steady release of nutrients. Using nano-fertilizers helps to reduce loss of nutrients while improving fertilizer-use efficiency of crops. The objective of this experiment was to study the effect of nano-chelated zinc, nano- chelated iron, and nano- chelated manganese on the some morpho-physiological traits and seed yield of chickpea under rainfed conditions.

MATERIALS AND METHODS

The experiment was conducted as a split plot experiment in a randomized complete block design with three replications. The first treatment tested three sulfur fertilizer levels in main plots and the second treatment involved three nano-chelated micronutrient fertilizers (nano- chated zinc, nano-chelated iron and nano-chelated manganese) in subplots. Field experiment was done using Deci chickpea cultivar Kakaie at Takab district in northwest of Iran during crop growing season of 2014–2015. Takab is located at an altitude of 1,765 m and is representative of upland semi-arid region. The sulfur fertilizer levels were S1: no application, S2: 15 Kg ha^{-1}, S3: 30 Kg ha^{-1} which were mixed with top soil and spread over the soil surface and incorporated into the top 10 cm of soil. The levels of nano-chelated micronutrient were Nano1: nano-chelated zinc, Nano2: nano-chelated iron, and Nano3: nano-chelated manganese which were applied at rate of 1 kg ha^{-1} at 30 and 60 days after sowing date. All of the nano-fertilizers used in this study were obtained from the Sepeher Parmis Company, Iran. Nano-fertilizers contained nano particles of zinc oxide, ferric oxide and manganese (II) oxide and morphological characterization of nano-ferric oxide was determined by scanning electron microscope (Fig. 1).

The climate of Takab region is identified as a cold semi-arid, its average annual rainfall was 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 the soil in the 0–40 cm layer is 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 usual 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. Seeds were 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 in the 2 × 2 m plots. Weeds were controlled frequently by hand weeding and there was no irrigation due to rain fed conditions. Vegetative growth period (VGP) and day to maturity (DM) was recorded for each plot and through the filed monitoring in interval of 2–3 days. Plants were harvested by hand at June and some morphological traits consist on 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 experimental plot. The 1,000 seed weight (TSW) was measured from ten random sample of each experimental plot. Seed yield (SY) and biological yield (BY) were determined by harvesting the middle three rows of each plot after avoiding border effects and harvest index (HI) was calculated according to the ratio of seed yield to biological yield. The two-way matrix of treatment × trait (TT) biplot model is generated according to Yan and Rajcan (29). Visual analysis of dataset via TT biplot was performed using GGEbiplot software (32) and all biplots presented in this paper are direct outputs of this statistical software. Up-to-date information on GGE biplot is available at http://www.ggebiplot.com.

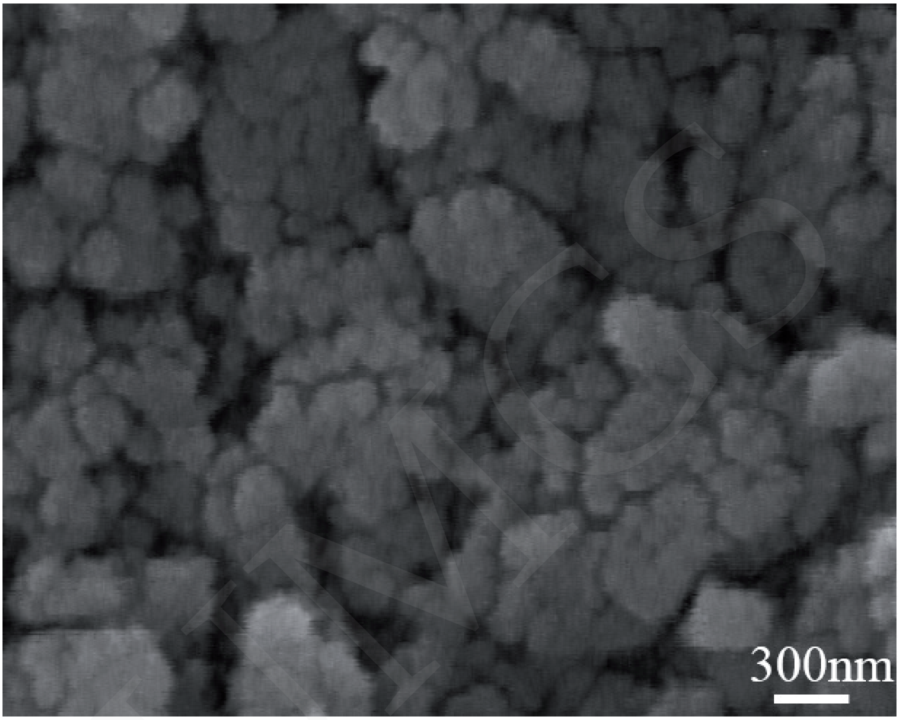

Fig. 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles of ferric oxide utilized for nano-chelated fertilizer

RESULTS AND DISCUSSION

During TT biplot model, variance for first two principal components, PC1 and PC2, were scored 56% and 18%, respectively, and cumulative variance of first two PCA was found 74%. The TT biplots showed the variation of the nine fertilizer treatments in terms of 13 traits. The TT (Fig. 2) identified five fertilizer treatments as best ones for single or multiple traits, and grouped the nine fertilizer treatments based on traits that make them potential performances. S3-Nano2 (30 kg ha^{-1} sulfur plus nano-chelated iron) was the highest performing fertilizer treatment for VGP trait (vegetative growth period), S1-Nano3 (0 kg ha^{-1} sulfur plus nano-chelated manganese) was the highest for EPP trait (number of empty pod per plant), and S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) was the highest for eleven traits (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 seeds per plant (NSP), and 1,000 seed weight (TSW), seed yield (SY), biological yield (BY) and harvest index (HI) (Fig. 2). The other vertex fertilizer treatments (S1-Nano2 and S2-Nano1)

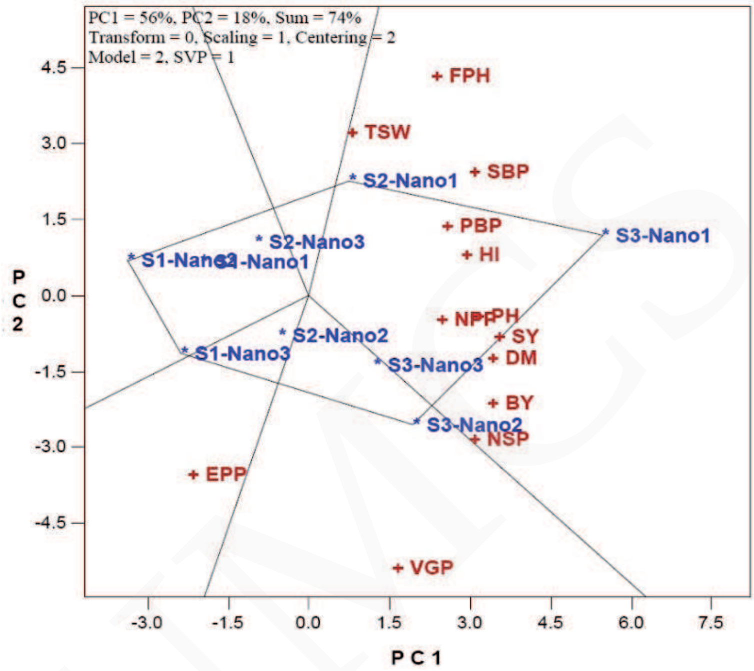

Fig. 2. Polygon view of TT biplot showing which fertilizer treatment had the highest values for which traits

were not the best in any of the measured traits. Therefore, it seems that for obtaining the best performance in most of the measured traits such as yield components and seed yield, application of S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) would be useful and it shows that both sulfur and zinc micronutrients are essential for chickpea production under rainfed conditions. Zn application provided a significant increase in seed yield and this increase was maintained in chickpea with the application of Zn under the field conditions (19). In a study conducted by Khan et al. (1998) on chickpea, it was reported that the Zn application created different effects on the measured traits but significantly improved seed yield. According to Islam et al. (11), there was an increase up to 12% in the seed yield of chickpea due to application of 30 kg S ha^{-1}. Also, Hussain (10) reported 15% increase in seed yield of soybean due to application of 30 kg S ha^{-1} under rainfed conditions.

The TT biplot of Fig. 3 displays the relationship of 13 traits of chickpea for 9 fertilizer treatments. In TT biplot, a vector drawn from origin to each trait facilitates the visualization of interrelationships among traits and the vector length of the trait measures the magnitude of its effects (30). The correlation coefficient between any two traits is approximated by the cosine of the angle between their vec-

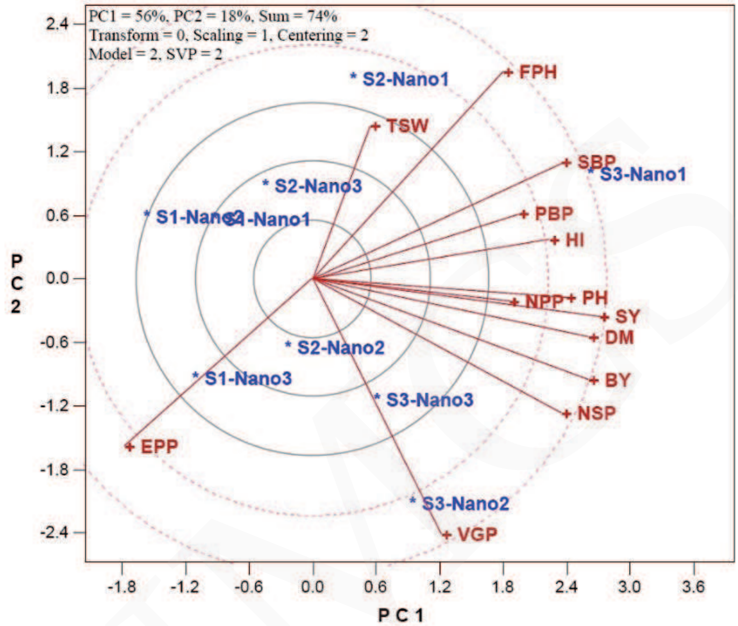

Fig. 3. Vector view of TT biplot showing the interrelationship among measured traits under different fertilizer treatments

tors (29). On this premise, two traits are positively correlated if the angle between their vectors is an acute angle (< 90°) while they are negatively correlated if their vectors are an obtuse angle (> 90°) (28). Across the 9 tested fertilizer treatments seed yield (SY) was positively associated with NPP, HI and DM (Fig. 3). However, the biological yield (BY) was positively associated with NSP and harvest index (HI) was positively associated with SBP and PBP traits. Number of empty pod per plant (EPP) was negatively associated with first pod height (FPH) and was negatively associated with HI, SBP and PBP (Fig. 3), because the vector trait of EPP made a 180 degree angle with the vector of the above mentioned traits which is indicating traits to be opposite in fertilizer treatments. A near zero correlation between TSW with BY and NSP, between VGP with HI, PBP SBP, and between VGP with EPP, as indicated by the near perpendicular vectors (Fig. 3). According to Jeena et al. (12), seed yield was significantly and positively correlated with number of primary and secondary branches, number of pods per plant, harvest index and biological yield. Likewise, similar results were reported by Thakur and Sirohi (25) and Kumar et al. (17). Most of the above findings can be verified from the original correlation coefficients (Table 1), but some others are not consistent with them because such discrepancies are seen due to the TT biplot explained lower than 100% (in present study, 74%) of the total variation.

Table 1. Simple correlation coefficients among chickpea traits under rainfed conditions.

	VGP	DM	PH	FPH	PBP	SBP	NPP	EPP	NSP	TSW	SY	BY
DM	0.60											
PH	0.43	0.79										
FPH	-0.39	0.36	0.54									
PBP	0.13	0.49	0.38	0.60								
SBP	0.05	0.69	0.69	0.81	0.83							
NPP	0.26	0.66	0.29	0.33	0.35	0.32						
EPP	0.19	-0.55	-0.30	-0.69	-0.68	-0.74	-0.62					
NSP	0.67	0.77	0.72	0.32	0.58	0.57	0.56	-0.24				
TSW	-0.22	0.21	0.25	0.29	-0.16	0.16	0.09	-0.30	-0.32			
SY	0.53	0.90	0.87	0.51	0.53	0.68	0.62	-0.43	0.82	0.28		
BY	0.68	0.93	0.71	0.33	0.58	0.61	0.76	-0.47	0.91	0.00	0.91	
HI	0.25	0.66	0.88	0.62	0.33	0.62	0.32	-0.28	0.55	0.54	0.88	0.61

Critical values of correlation $P<0.05$ and $P<0.01$ (degrees of freedom = 7) are 0.67 and 0.75, respectively.
Abbreviations are: vegetative growth period (VGP), 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), 1000 seed weight (TSW), seed yield (SY), biological yield (BY) and harvest index (HI).

The TT biplot has been used in assessing the repeatability of entry or treatment using model-2 of the biplot (31) and ideal type of entries has been identified as ideal or core entries which is very important in a research program for selection exercise when selection is done only at a single treatment. In other words, the concept of ideal treatment is the situation that is most favorable treatment among all treatments. It has been shown that the distance between one treatment and the ideal fertilizer is a more repeatable parameter to evaluate the treatment performance. In a TT biplot, the center of the concentric circles on the average tester coordinate indicates the ideal treatment (Fig. 4), 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. Therefore, the S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) might be applied for selecting superior traits and it can be considered as the candidate treatment. Moreover, the performance of various traits by 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) combined fertilizers were observed above the average while the other treatments (S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano2 and S2-Nano3) were below

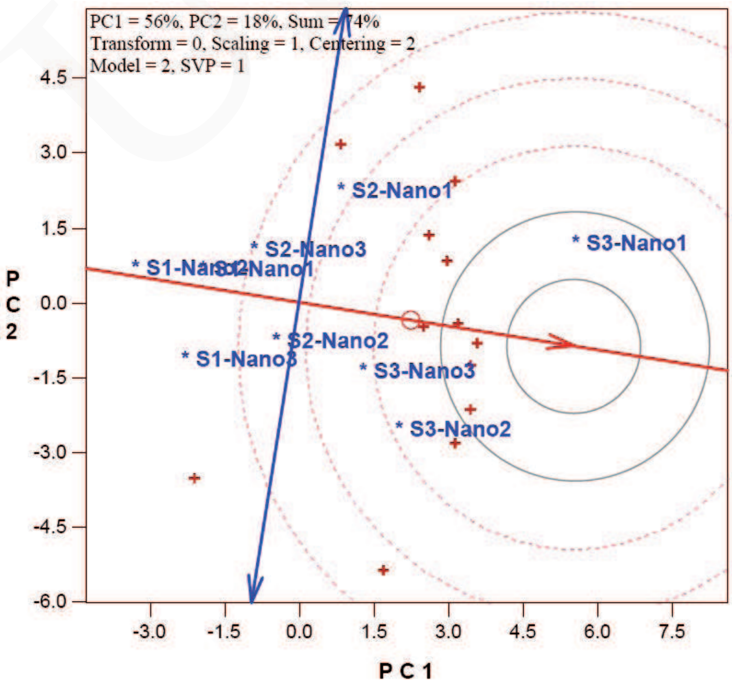

Fig. 4. Ideal entry view of TT biplot, showing the relationships of different fertilizer treatments with ideal entry

average (Fig. 4). Therefore, it seems that application of high amounts of sulfur plus nano-chelated zinc followed by to nano-chelated manganese as well as nano-chelated iron which are useful in achieving to favorable micronutrient fertilizer application in chickpea production under rainfed conditions in semiarid environments. This study produced results which corroborate the findings of Ghasemi-Fasaei et al. (6) and Pahlavan Rad and Pessarakli (21).

In addition to the results of the traditional method of analyzing entry × tester (treatment × trait) data, biplot provides information on the effectiveness of the testers with the view of identifying the ideal (best) tester or trait. An ideal trait should have the largest vector of all traits (i.e., be most discriminating) and have zero projection onto the ATC (average tester coordinate) ordinate (i.e., be most representative of traits). Thus the closer a trait's marker was to the ideal trait, the better it was. Figure 5 displays the performance of the traits in relation to their crosses with the treatments. The TT biplot showed that seed yield (SY) following to PH, DM, BY, HI and NPP had the highest discriminating ability and they were the most representative. Therefore, it seems that seed yield as the final target trait of producers has the ability of discrimination among different treatments.

The best fertilizer treatments for obtaining high seed yield (SY) could be found in the TT biplot of Figure 6 which is a vector-view function and shows

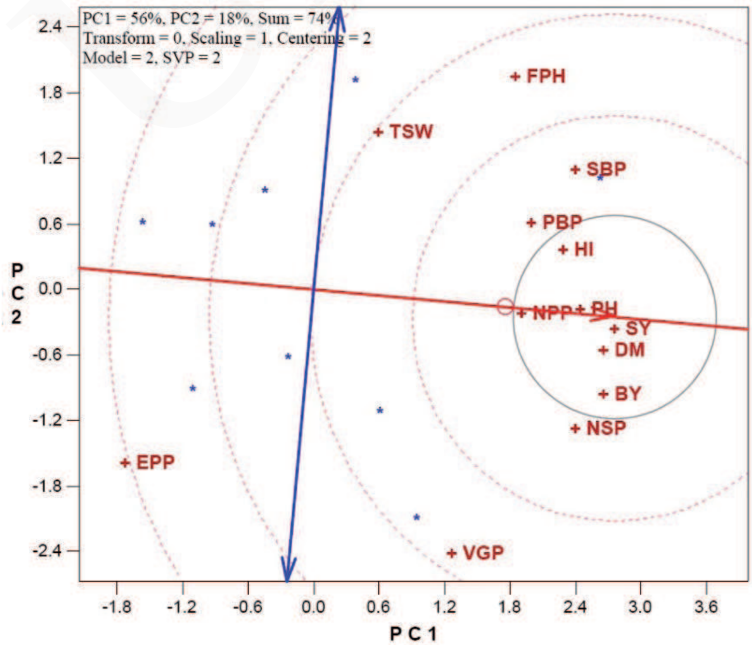

Fig. 5. Ideal tester view of TT biplot, showing the relationships of different traits with ideal tester

fertilizer treatments that have close association with a target trait among other traits. According to this biplot of Figure 6, S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) treatment was the best fertilizer treatment suitable for obtaining high seed yield. Thus, application of this treatment combination is expected to lead to improved target trait (seed yield) under rainfed growing conditions in semi-arid region. This suggests that application of zinc nano-chelated fertilizer plus sulfur will not only result in the development of high seed yield but also improved the other desirable agronomic traits which are associated to seed yield. Bala et al. (3), reported that the beneficial role of nano-fertilizer application in germination and growth of chickpea is demonstrated. Furthermore, Amirnia et al. (2) have emphasized the positive effects of some micronutrient and macronutrients nano-fertilizers (iron, phosphorus and potassium) on saffron (*Crocus sativus* L.) production. Liu et al. (18) reported that nano-particles' application was safe for wheat production and has some economic benefits. Kharol et al. (16) 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.

Our results indicate that the polygon view as well as vector view of TT biplot are best to visualize the interaction pattern between treatments and traits, provided the biplot should explain a sufficient amount of the total variation. Among the

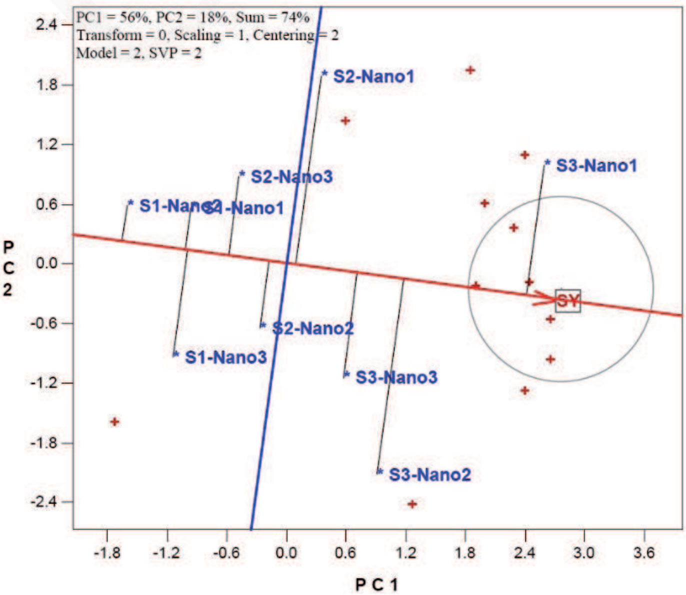

Fig. 6. Vector view of TT biplot, showing the relationships of different fertilizer treatments with seed yield (SY)

multivariate analysis methods, TT biplot has the widest applicability in the analysis of data because the interpretation of biplot analysis is more extensive with wider applicability than the conventional statistical methods (27). The results of a TT biplot can, however, be deficient if the PC1 and PC2 account for only a small proportion of the total variation but it does not allow a serious statistical testing of hypothesis, the reliability of its results has become questionable in the literature. However, our results have been found to be consistent with those of ANOVA, correlation, regression and multivariate statistical methods. One of the most important applications of nanotechnology in agriculture is Nano formulation of fertilizers. Plant nutrition with nano-fertilizer in comparison with the conventional fertilizers have huge difference in the accuracy, smart nature, effectiveness, cost for operation, ease of construction and many others. There is awareness about the risks of consuming rather than the benefits of the technology and in spite of all these drawbacks there is continuous research carried out in nanotechnology, there will be a day which will come in the near future for an accepted nanotechnology.

CONCLUSIONS

Our investigation indicated that application of nano-chelated Zn plus sulfur increase chickpea's seed yield, primarily due to an increase in the days to maturity and plant height, secondary due to an increase in the number of pods per plant, biological yield and harvest index, tertiary due to an increase in the number of seeds per plant and primary branch per plants. High amounts of sulfur (30 kg ha^{-1}) and nano-Zn fertilizers following nano-Fe and nano-Mn fertilizers can cause a significant increase in seed yield of chickpea cultivated in semiarid region under rainfed conditions.

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