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Treatment by Trait Biplot Analysis of Organic Manure and Nano-Fertilizers on Sunflower Production

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Abstract: This study was performed to study the effects of farmyard manure (zero (M1), 20 (M2) t ha^{-1}) and nano-fertilizers [control (n1), Mn (n2), Fe (n3), Zn (n4)] on sunflower. Traits days to 50% flowering, days to maturity, leaf number, leaf area, plant height, achene yield and harvest index were measured. Treatment by trait (TT) analysis using biplot analysis was used to determine which treatment was best and for what trait which were generated using the standardized values of the traits means. Results showed that the first two principal components (PC1 and PC2) were used to create a two-dimensional TT biplot that accounted percentages of 94% of sums of squares of interaction. The most important vertex treatment in polygon of TT biplot was M2n4 (application of 20 tonnes ha^{-1} manure and Zn nano-micronutrient) which indicated high performance in leaf number, leaf area, plant height, achene yield and harvest index. The identification of ideal treatment, the treatment that is most favorable treatment among all treatments, showed that the M2n4 might be used in selecting superior traits and it can be considered as the candidate treatment. Finally, nano-fertilizer could increase crop yields and improve the efficiency of manure application. The results of this investigation showed that application of nanoparticles may alleviates the adverse environmental factors and improve the sunflower performance and the integrated application of organic manure and nano-micronutrients is more effective.

Keywords: achene yield, farmyard manure, nano-micronutrient, zinc oxide

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Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crops and most of its cultivation lies in arid and semiarid regions, due to its wide adaptability. It is responsive to agronomic practices especially nutrition management in accordance with local soil factors but it is still facing low production which might partly be attributed to poor soil fertility and inappropriate nutritional management (Ryan *et al.*, 2012). It has been estimated that world production of sunflower seed is exceeded 41.4 million tonnes from an area of 25.2 million ha land with 16435 hg ha^{-1} (1.6 t ha^{-1}) average mean yield while the annual production of sunflower in in Iran is near to 78,000 tones which achieved from 70 thousand ha with 11143 hg ha^{-1} (1.1 t ha^{-1} or about 32% lower than global mean yield) average mean yield (FAOSTAT, 2014). However, the average yield of sunflower in semi-arid regions is relatively low and the potential of new high-yielding varieties can be fully exploited by the application of necessary inputs at suitable growth stages. Soil of semi-arid regions of are tilled, it is low in organic matter content and consequently have weak structural stabilities (Shirani *et al.*, 2002). Farmyard manure is rich in nutrients and can supplies all major macronutrients essential for plant development, as well as micronutrients and their application increase organic matter content in soil. Organic matter serves as a reservoir of nutrients and water in the soil and aid for reducing compaction and surface crusting, it can increase nutrients availability and improves soil physical properties significantly (Mwahija, 2015).

Micronutrients are required by plants throughout life in small quantities to organize a range of physiological functions, plant development and crop production. Among the micronutrients, manganese (Mn), iron (Fe) and zinc (Zn) are essential for crop growth and production. Mn is playing a key role in photosynthesis, lignin synthesis and stress tolerance, Fe is involved in the production of chlorophyll, photosynthesis, mitochondrial respiration, hormone biosynthesis, and production of reactive oxygen species, Zn is a necessary component of various enzyme systems for energy production, protein synthesis, energy production and growth regulation (Alloway, 2008; Marschner, 2012). Although the chemical fertilizers have some advantages, their performance needs a standard basis of chemical and physical condition soils while nano-fertilizers deals with the active nutritional ingredients at nano-scale dimensions which enables them to partially synchronize the nutrients release with crop requirements (De Rosa *et al.*, 2010). Nanotechnology is a novel science that owing to high ratio of surface area to volume, nano-materials exhibit novel and improved properties and functions and the nano-fertilizers have a slower release compared to the conventional fertilizer application (Raliya and Tarafdar, 2013). They will prevent undesirable nutrient losses to environment (soil, water and air) via direct delivery to plants, and avoiding the interaction with soil, microorganisms, water, air and other factors (De Rosa *et al.*, 2010).

Zhang *et al.* (2006) investigated the effects of slow release fertilizers cemented and coated by nano-materials and reported that nano-composites were safe for seed germination and seedling growth because they provide an efficient tool for regulated and timely delivery of nutrients to crops. The application of zinc nano-fertilizer on pearl millet improved some properties such as chlorophyll content, total soluble leaf protein, plant dry biomass and the yield was improved by about 40 % due to application of zinc nano-fertilizer. (Tarafdar *et al.*, 2014). Efficiency of nano-fertilizer under different organic manure levels should be investigated clearly and this will creates the angle of vision for the nano-fertilizer application in semi-arid region for sunflower production. In Iran, Mn, Fe and Zn deficiencies are nutritional disorders in most of the crops grown in dry land conditions, and these nutrients are meaningfully involved in yield production. Since there have been little studies on interaction of nano-fertilizers and farm-yard manure, the present investigation was performed.

Material and methods

Hybrid Iroflor of sunflower was cultivated in Maragheh (46° 16′ E and 37° 23′ N with an altitude of 1485 m) in northwest of Iran during 2015–2016 growing season. The experiment was established on a silty loam soil with pH 8.07, organic matter content 0.92 %, total nitrogen 0.13 %, Caco3 17 %, electrical conductivity (EC) 0.8 ds m^{-1}, phosphorous 15.31 ppm and potassium 820 ppm (0 to 60 cm depth). The trial was performed in a split-plot experiment according to randomized complete block design with three replications keeping farmyard (zero (M1) and 20 (M2) tonnes ha^{-1}) in main plots and nano-chelated micronutrient [control (n1), Mn (n2), Fe (n3), Zn (n4)] in sub plots. Chemical properties of farmyard manure included 31.4 % of organic matter, 1.65 % of total N, 850 ppm of available P and 3096 ppm of available K, pH 8.23, Ec 3.1 ds m^{-1}. After tillage operations, well rotten farmyard manure was applied as per the treatment and thoroughly mixed into the top soil. Each plot was 4 m long and 3 m wide. Row to row and plant to plant (on the row) distances were 60 cm and 20 cm, respectively. Sunflower was sown on April 3 and harvested on 21th August. Nano chelate fertilizers were obtained from the Sepeher Parmis Company, Iran, which contained zinc oxide, ferric oxide and manganese (II) oxide nanoparticles.

Nano-chelated fertilizers were applied at rate of 1 kg ha^{-1} through fertigation 30 and 60 days after sowing date.

Days to 50 % flowering (DF) and days to maturity (DM) were recorded. Leaf number (LN) and leaf area (LA) were measured during the initiation of reproductive stage when terminal bud forms a miniature floral head rather than a cluster of leaves (R1). Phenology and plant development was evaluated according to Schneiter and Miller (1981). Plant height (PH) was measured from the soil surface to the top of the uppermost plant organ. Plants harvested at physiological maturity (R9) when the bracts became yellow and brown and achene yield (AY) was determined. Harvest index (HI) indicates the ratio of achene yield to biological yield (stalk + capitulum). Treatment by trait (TT) analysis using biplot analysis (Yan and Kang, 2003) was used to determine which treatment was best and for what trait which were generated using the standardized values of the traits means. The model for a GGE (G + GE) biplot, based on singular value decomposition of the first two principal components (PC1 and PC2). The biplot analysis was based on Model 2 (i. e., dataset was not transformed (Transform = 0) within-trait standard deviation standardized (Scale = 1), and trait-centred (Centering = 2). The polygon-view was based on treatment-focused singular value partitioning (SVP = 2), while the vector views were based on the trait-focused singular value partitioning and is, therefore, appropriate for visualizing the relationships among traits and genotypes. This model was used to generate a biplot of "which-won-where" and for the analysis of the relationship between the treatments and traits. The biplot model equation for treatment by trait interaction biplot analysis is presented as follows:

$$(Y_{ij} - \mu - \beta_j) = \lambda_1 g_{i1} g_{1j} + \lambda_2 g_{i2} g_{2j} + \sum_{ij}$$

Where:

Y_{ij} is the genetic value of the combination between genotype i and trait j;

μ is the mean of all combinations involving trait j;

β_j is the main effect of trait j;

λ_1 and λ_2 are the singular values for principal component PC1 and PC2;

g_{i1} and g_{i2} are the PC1 and PC2 eigenvectors, respectively, for genotype *i*;

e_{1j} and e_{2j} are the PC1 and PC2 eigenvectors, respectively, for trait *j*;

d_{jis} the phenotypic standard deviation; and

Σ_{ij} is the residual of the model associated with the combination of genotype *i* and trait *j*,

The analyses were conducted and biplots generated using the "GGEbiplot" software (Yan, 2001).

Results and discussion

The first two principal components (PC1 and PC2) explained 94 % of total variation (Figure 1). In order to distinguish meaningful groups of testers (traits), repeatable patterns among traits have to be demonstrated through TT biplot analysis and showed that the "which-won-where" view of biplot and the outmost treatments (five in this case) formed a five-side polygon. This biplot was divided into five sectors delimited by the lines perpendicular to each side of the polygon and the measured traits fell into the three sectors. For traits within a sector, the nominal "winner" is at the vertex and so, M1n1 and M2n1 were the wining treatments only for a single trait, days to 50 % flowering (DF) and days to maturity (DM), respectively (Figure 1). However, M2n4 (application of 20 tonnes ha^{-1} manure and Zn nano-chelated micronutrient) was the winning treatments in the sector containing the leaf number (LN), leaf area (LA), plant height (PH) achene yield (AY) and harvest index (HI). The wining treatments, M1n3 and M2n3 were not the wining treatments in even one of the measured traits (Figure 1). The results shown in Figure 1 suggested that there might be distinct groups of traits in compliance with the large magnitude of treatment by trait interaction and the high value of PC1 and PC2 contributions to the total sum of squares in TT biplot. Also, effect of Zn nano-chelated micronutrient plus application of 20 tonnes ha^{-1} manure were demonstrated in most of sunflower traits and so this treatment combination could be recommended for sunflower producers. The results confirmed that manure addition to soil and application of Zn increased performance

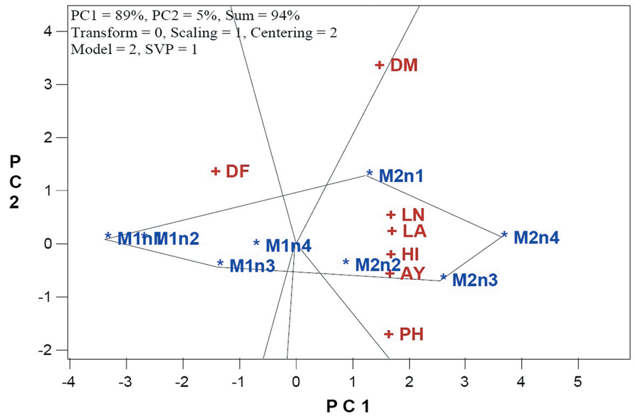

Figure 1: Polygon-view of treatment by trait (TT) biplot showing which treatment had the highest values for which traits.

of target traits. This study advanced our knowledge on possible agronomic practices to biofortify sunflower and showed that combined addition of manure and Zn fertilizer can considerably raise yield.

Summary of the associations between traits provides Figure 2 which shows the lines connecting the biplot origin with the markers for the traits are called trait vectors and the angle between the vectors of two traits is related to the association coefficient between them. The cosine of the angle between the vectors of two traits approximates the association coefficient between them (Yan and Rajcan, 2002), and based on the cosine of angles of traits vectors, there were positive correlation among LN, LA, PH, AY and HI (Figure 2). Also, there was near zero correlation between DM with PH while the presence of wide obtuse angles i. e. strong negative correlations among the traits between PH with DM is indication of strong cross-over TT interactions (Yan and Tinker, 2006). Our results are in agreement with the work of Chikkadevaiah *et al.* (2002), Gjorgjieva *et al.* (2015) and Jockovic *et al.* (2015).

As depicted in Figure 3 the single-arrowed line called average–tester coordination abscissa points to higher performance across traits to identification of the ideal treatment which has high performance in most or all measured traits. When traits are ranked across treatments, it should be done with respect to an ideal trait that lies on average–tester coordination abscissa (absolutely stable) in the positive direction and has a vector length equal to the longest vector of the

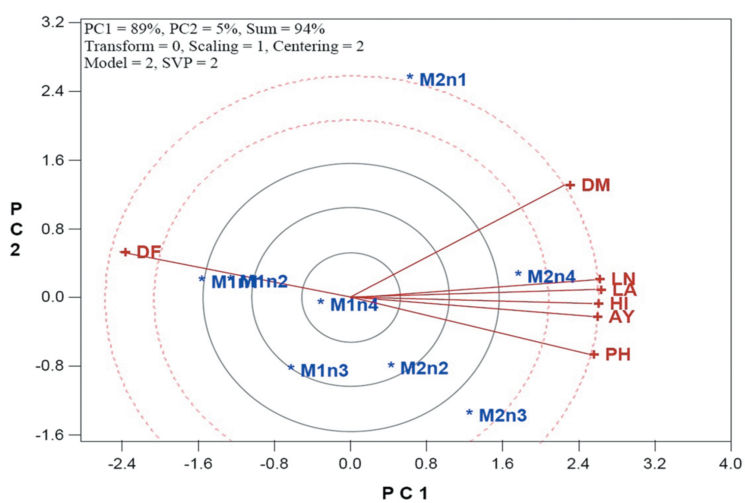

Figure 2: Vector view of treatment by trait (TT) biplot showing the interrelationship among measured traits under different treatments.

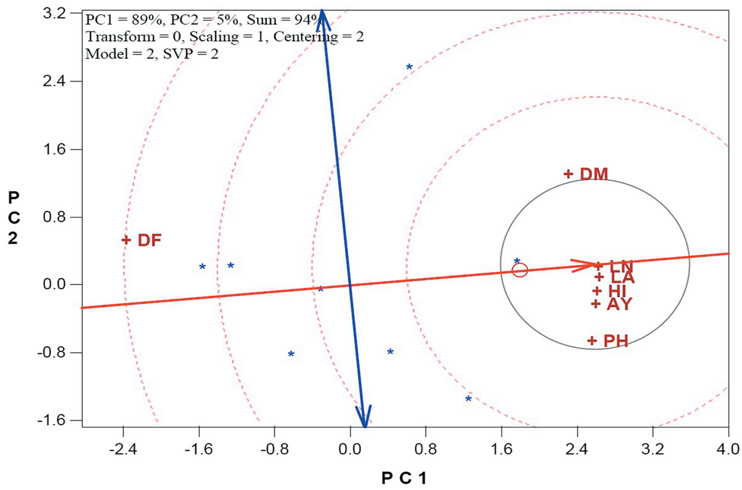

Figure 3: Ideal tester view of treatment by trait (TT) biplot, showing the relationships of different traits with ideal tester.

traits on the positive side of this abscissa i. e., highest performance, therefore, traits which are closer to “ideal trait” are more desirable than others (Yan and Tinker, 2006) and thus, LN, LA, HI, AY, PH and DM were high performance with consistent performance across the treatments (Figure 3). TT biplot instability distance from the ideal entry is the measure of determination of the best entry or treatment and among the four highest performing entries, M2n4 following to M2n1 and M2n3 treatments were identified as the most favorable treatment (Figure 4). Furthermore, TT biplot analysis indicated that manure application plus nano-fertilizers performed better than M1n1 (no manure plus no nano-fertilizer) treatment (Figure 4).

Results of current study showed that most traits improved by both manure and nano-micronutrient fertilizers. The findings of the current study are consistent with those of El-Ghamry *et al.* (2009) who found that farmyard plays an important role for supplying some essential plant nutrients. In this study both sink and source organs were affected by nutrient managements because leaf number and leaf area affected by both organic and inorganic fertilizers. This also accords with our earlier observations, which showed that an appropriate nutrient management considerably increased the source size in safflower (Janmohammadi *et al.*, 2016). Increase of achene yield under fertilizer application can be attributed to the improved supply of photoassimilates resulting from the abundance of essential elements which are utilized for enlargement of the

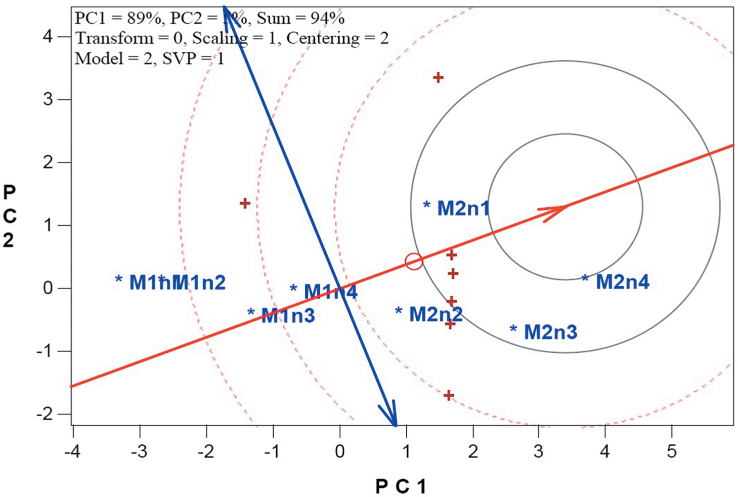

Figure 4: Ideal entry view of treatment by trait (TT) biplot, showing the relationships of different treatments with ideal entry.

sink cells (Dordas and Sioulas, 2008) and it is encouraging to compare this finding to that found by Janmohammadi *et al.* (2014), who found that the manure application increased the lentil yield through the improvement of the both sink and source strength.

It seems that farmyard manure could bring a suitable condition for plant growth that resulted in accelerated vegetative growth, long reproductive period and greater green area duration. Farmyard manure is known to enhance soil fertility, which in turn invigorate vegetative growth and extend time to maturity (Figure 5). The present findings seem to be consistent with other research which found that combination of the organic sources with inorganic nutrients gives higher oil content rapeseed and Indian mustard (Tripathi *et al.*, 2011; De and Sinha, 2014). The most interesting finding was that application of nano-fertilizers under non-manured conditions could not considerably affected the achene yield components as indicated in chemical composition of manure, its application can supply considerable amounts of different essential macronutrients and small amounts of micronutrients for crop plants. Despite the positive effects of nano-micronutrient fertilizers, a supplementation of nano-fertilizers with a considerable proportion of organic manures is necessary to boost up sunflower production in semi-arid region.

Nanoparticles have unique physicochemical properties and it seems that they have potential to mitigate the adverse effects of heat and drought stress on

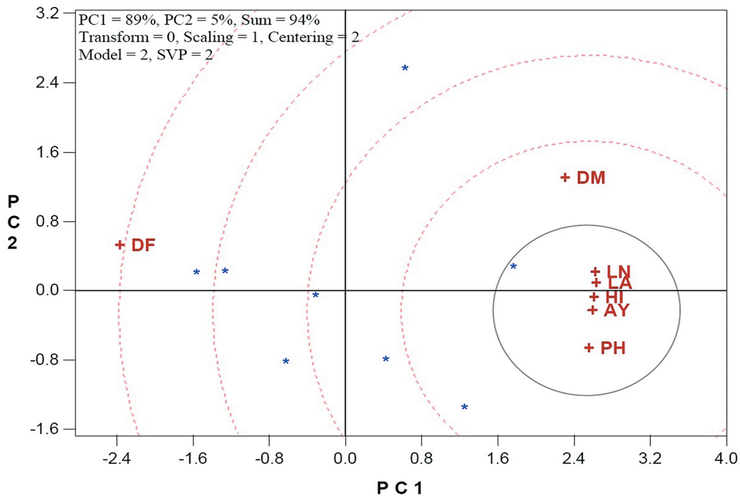

Figure 5: Vector view of treatment by trait (TT) biplot, showing the relationships of different treatments with achene yield (AY).

the growth and physiology of sunflower. Effectiveness of nanoparticles strongly depends on their concentration and varies from plants to plants, therefore effects of different concentrations and the interaction nanoparticles with cellular process needs further evaluation and may provide a more comprehensive interpretation. No doubt, nanotechnology is an evolutionary science and has introduced novel applications in the agriculture due to their unique properties and a number of researches have been done on the different effect of nanoparticles on crops, but research focusing on the beneficial effects of nanoparticles on crop remains incomplete. This research reveals that the research on nano-titanium dioxide and nano-silica particles on sunflower, is in the beginning; more rigorous works are needed to understand physiological mechanisms of plants in relation to these nanoparticles as well as studies are needed to explore the mode of action of nanoparticles, their interaction with biomolecules.

Conclusions

Application of Zn nano-fertilizer following Fe and Mn nano-fertilizers plus manure considerably enhanced the yield and yield components of sunflower performance in semi-arid region. The biplot analysis could help to visualize the associations among treatments, traits and interaction between them and so was an efficient statistical tool for interpreting such dataset. It is noted that in semi-arid region with relatively poor soil, nano-fertilizers should not be applied alone, rather in combination with organic fertilizers to obtain the maximum yield.

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