EFFECTS OF METAL OXIDES AND UREA FERTILIZER ON AGRONOMIC TRAITS OF SAFFLOWER

M. Janmohammadi, M. Fattahi, N. Sabaghnia, M. Nouraein

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

In Mediterranean semi-arid regions major constraints impinging on agricultural development are deficiency of water and nutrient depleted soils. The problem of enhancing crop yield in these areas is widely distinguished as a challenge. In order to evaluate the integrated application of urea fertilizer (0, 160, and 320 kg ha^{-1}) and nano-metal oxide (Fe_2O_3, ZnO, and CuO) on safflower growth performance, a field experiment was carried out in the Baneh district, a semi-arid region in Western Iran. Urea fertilizer significantly increased the plant height (11%), canopy width (8%), ground cover percentage (6%), plant dry weight (35%), number of secondary branches (16%), seeds number per head (19%), and total seed yield (38%). However, the higher application of urea fertilizer resulted in a significant decrease in wrinkled seed percentage (3.25%), harvest index (2.62%), and thousand seed weight (2.67%). Also, nano-metal oxides significantly affected morphological traits and yield components. Mean comparison revealed that the best performance was obtained by the integrated application of 320 kg urea fertilizer and nano-Fe or nano-Zn. Overall, the present research highlighted the necessity of balanced and integrated application of macronutrients and micronutrient fertilizers for sustainable safflower production in semi-arid regions of Western Iran.

copper, essential micronutrients, morpho-physiological traits, nutrients interaction, yield components

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INTRODUCTION

Safflower (*Carthamus tinctorius* L.), an annual plant from Asteraceae family, is a highly branched, herbaceous, thistle-like, warm season broadleaf plant which can be considered as oilseed and forage crop (Oelke et al., 1992). This crop can be grown throughout the semi-arid regions of temperate climate, which is partly due to the deep-root system of safflower which can tolerate water-deficient condition (Dordas, Sioulas, 2008). Major producers of safflower are India, the United States, Kazakhstan, Argentina, and Mexico. The total safflower seed production in 2014 was about 734 000 t from 937 000 ha (http://www. fao. org/faostat/en/#data/QC). However, Safflower seemingly still has not achieved the deserved position in the oil production Safflower can be grown in rotation with other crop species. Winter wheat is dominant crop in semi-arid areas and newly introduced crop should have substantial agronomic benefit to the cropping system and also enhance the farmers' economic position (Johnston et al., 2002). Due to the specific characteristics of safflower and its relative tolerance to drought and heat stress it seems to be a good choice for these areas.

Soil has a key role in determining an agro-ecosystem's productivity. Sustainable productivity of a soil primarily depends upon its ability to supply essential nutrients to the growing plants (Kumar, Babel, 2011). Although the deep-root system of safflower enables it to absorb water and other essential nutrients from the depth of the soil, very low soil fertility considerably decrease the plant growth in semi-arid regions. However, nutrient management has not been well investigated in this crop. An outstanding feature of the soils in these regions is their inherently low fertility, with negative plant-nutrient balance. Moreover, some other socio-economic factors may encounter safflower production with a serious challenge. These factors include small and fragmented land holdings, a weak agricultural educational and technology infrastructure, poor credit facilities, and low inputs of fertilizers and other agrochemical inputs (Ryan, 2008). It seems that there are obvious simplifications in the nutrient management concepts for safflower production systems, such as neglecting micronutrients requirements, interaction of macro and micro nutrients, the effect of micronutrients on qualitative characteristics, formula and chemico-physical properties of utilized fertilizers, method of fertilizing (foliar spray, soil application), and the fertilizer schedule.

Nitrogen is one of the imperative nutrients for plant growth and its availability limits the productivity of most cropping systems. Since nitrogen is used in amino acid and protein structures, its roles in cellular process are undeniable. Nitrogen significantly affects dry matter production by influencing leaf area expansion and leaf area duration as well as photosynthetic efficiency (Yang et al., 2006; Shahrokhnia, Sepaskhah, 2017). Establishing a criterion for N is as important as for water. Nitrogen fertilizer often gives the least profitable returns of the essential nutrients because of the large amounts required and high price per unit (Wiedenhoeft, 2006). Also, micronutrients or trace minerals are used by the plant in small quantities, nevertheless are essential for plant growth and survival. Among micronutrients, zinc is highly regarded by the researchers. Zinc is a key constituent of many enzymes and proteins. It plays an important role in a wide range of processes, such as growth hormone production and internodes elongation. Zinc deficiency was found to be a plant nutritional problem throughout the Mediterranean semi-arid regions (Cakmak, 2008), resulting in substantial losses in crop yields and human nutritional health problems. Research has demonstrated that high pH in calcareous soil noticeably reduces availability of this element in semi-arid region (Alloway, 2008). Likewise, copper is a micronutrient substantially involved in electron transfers in energy exchange reactions within the cell, due to its variable oxidation status. It is a component or activator of some enzymes (Wiedenhoeft, 2006; Udeigwe et al., 2016). Furthermore, iron is the third most limiting nutrient for plant growth and it is necessary for photosynthesis as it is involved in chlorophyll synthesis, and it is essential for the maintenance of chloroplast structure and function (Rout, Sahoo, 2015). Iron is required for the biosynthesis of cytochromes and other heme molecules, including chlorophyll, the electron transport system, and the construction of Fe-S clusters (Hänsch, Mendel, 2009). Reportedly millions of hectares of farmland soil worldwide lack sufficient amounts of trace elements. Moreover there is a lack of sufficient and comprehensive information about safflower response to integrated application of nutrients. Therefore, studies on the circulation and balance of trace elements in ecosystems have attracted increasing scientific interest.

However, research has demonstrated that chemical fertilizers are considered as double-edged swords, which on the one hand improve the quantitative aspect of crop production but on the other hand disrupt the soil mineral balance and may increase the risk of environmental pollution (Solanki et al., 2015).

However, in the last few years, some researchers tried to examine the potential of nano-biotechnology to improve micronutrients use efficiency and strategies resulting in the design and development of efficient new nano-fertilizer delivery platforms for use at the farm level (Naderi, Danesh-Shahraki, 2013; Pereira et al., 2015). Despite recent progress in understanding some environmental effects of micronutrient fertilizers, little research is available about the influence of integrated application of nitrogen and nano-metal oxide particles on the yield of safflower in the semi-arid region and conflicting results are often reported. However, due to the relative infancy of the field of nano-micronutrient fertilizers inputs, it is particularly important to understand their fate and effects under variable nitrogen application level. Therefore, this experiment was conducted to study the effect of integrated application of nano-particles of Zn, Fe, Cu and urea on growth and yield components of safflower under well irrigated condition.

MATERIAL AND METHODS

This study was designed to determine the effects of nano micronutrients and urea fertilizers on the growth of safflower in the Baneh region during the 2014–2015 growing seasons. Baneh is located in the west part of the Kordestdan district and situated

Fig. 1. Geographical location of the investigated area

Table 1. Effect of urea fertilizer and metal oxide nano-particles on some morphological traits of safflower (*Carthamus tinctorius* L.)

	PH	FBH	CW	SD	PDW	NSB	NSH	NTH	GCP	WSP
Urea fertilizer	**	NS	*	NS	**	*	*	NS	*	**
non	59.75^{b}	32.02^{a}	28.31^{b}	9.22^{b}	42.80^{c}	7.44^{b}	8.66^{b}	10.22^{a}	69.44^{b}	11.73^{a}
160 kg ha^{-1}	64.06^{ab}	30.48^{a}	29.13^{ab}	11.11^{a}	53.20^{b}	8.33^{ab}	10.33^{a}	10.88^{a}	70.55^{ab}	8.86^{b}
320 kg ha^{-1}	68.47^{a}	27.13^{a}	32.04^{a}	10.66^{ab}	62.93^{a}	9.00^{a}	10.44^{a}	11.66^{a}	76.66^{a}	8.48^{b}
	nano-metal oxide									
	**	*	**	**	*	**	**	*	**	**
Fe_2O_3	69.65^{a}	33.88^{a}	32.06^{a}	13.88^{a}	55.06^{a}	9.33^{a}	11.22^{a}	12.11^{a}	77.66^{a}	8.61^{b}
ZnO	65.02^{a}	30.17^{ab}	31.11^{a}	9.00^{b}	55.60^{a}	8.66^{a}	9.66^{b}	10.77^{ab}	73.33^{a}	8.93^{b}
CuO	57.62^{b}	25.75^{b}	26.31^{b}	8.12^{b}	48.26^{b}	6.77^{b}	8.55^{b}	9.88^{b}	65.66^{b}	11.54^{a}
U × N	NS	NS	NS	**	NS	NS	**	*	NS	NS
CV	7.26	22.41	10.83	18.06	12.59	13.66	13.87	18.19	9.12	16.12

PH = plant height at maturity (cm), FBH = first branch height (cm), CW = canopy width (cm), SD = stem diameter (mm), PDW = plant dry weight (g), NSB = number of secondary branches, NSH = number of heads in secondary branch, NTH = Total number of heads in plants, WSP = percentage of wrinkled seeds, GCP = ground cover percentage, U = urea fertilizer, N = micro-nutrient, NS = not significant

*significant at 5% level of probability, **significant at 1% level of probability, CV = coefficient of variance; mean values of the same category followed by different letters are significant at $P \leq 0.05$ level

1610 m a.s.l. between 35°59′ N latitude and 45°53′ E longitude. The climate of the area is typically semi-arid. Rainfall and temperature are the two main elements of the climate. Rainfall in this region is seasonal, erratic, and not properly distributed and it varies between 350 to 550 mm annually, mostly occurring in the period November–April. Maximum summer temperatures range from 28 to 48°C, winter is cold with minimum temperatures from –8 to 10°C. For Baneh location see the map in Fig. 1.

During the first year the field was abandoned as fallow and in autumn it was plowed by moldboard. The experiments were conducted as a factorial consisting of 2 factors (3 × 3) based on a randomized complete block design with 3 replications. The effects of 3 levels of urea fertilizers (0, 160, and 320 kg ha^{-1}) and 3 types of nano-metal oxides (Fe_2O_3, ZnO, and CuO) were evaluated under well-watered (non-stressed) conditions in a loam soil. Nano metal oxides were obtained from the Nano-Pishgaman Company, Iran. The synthesized nano particles were characterized morphologically by transmissionelectron microscope (Zeiss EM 10 C, Merck, Darmstadt, Germany). Urea fertilizer was purchased from Razi Petrochemical Company, Iran. The spring safflower (Carthamus tinctorius L.) cultivar 'Goldasht' was used in the experiment. Fertilization schedule has a significant effect on crop yields. Proper timing of the fertilizer application reduces nutrient losses, increases nutrient use efficiency, and prevents damage to the environment. Both fertilizers were applied as split utilization. Nano-metals were applied at the rate of 400 g ha^{-1} during the planting and vegetative and reproductive growth.

Urea fertilizer was applied as top dressing during three growth stages: planting, vegetative (stem elongation 1 visibly extended internode) and reproductive growth (beginning of capitulum formation, still enclosed by leaves). In each treatment 7 rows of safflower were sown. The rows were 40 cm apart with the plant to plant distance of 10 cm. A sprinkler system was applied for irrigation. The first irrigation was applied 1 day after sowing, and the second one was 2 weeks later. The plots were irrigated to 70% of field capacity. A water counter was used to record the volume of water applied. Water use at each irrigation interval was determined based on pre-irrigation soil moisture and about 5200 m^{3} water per ha was delivered.

Groundcover was determined as the amount of plant material (dead or alive) that covered the soil surface. It was expressed as a percentage through visual assessment; 100% groundcover means that the soil cannot be seen while 0% groundcover is bare soil. The average canopy spread is the average horizontal width of the plant canopy, taken from right to left as one moves around the plant. Groundcover and canopy spread was measured during the flowering stage (BBCH = 65; 50% of florets open in flowers on main shoot). Plant characters measurements included: stem diameter (SD), canopy width (CW), and plant dry weight (PDW) were carried out under filed condition in maturity stage. Plant height (PH), number of heads per plant (HP), number of seeds per head (SH),thousand seed weight (TSW), and head diameter (MHD) were determined using ten randomly selected plants from each plot. To determine the seeds and biological yield, plants were harvested from 2 m^{2} after eliminating two side rows of each plot and 0.5 m from both ends of central rows. TSW was determined by measuring the weight of 500 seeds from each plot multiplied by 2. The harvest index was calculated as seed yield/biological yield × 100.

Data were subjected to the analysis of variance (ANOVA) and means were compared (LSD test, $P < 0.05$) using the SAS 9.3 (2002) and MSTAT-C software packages. Correlation and path analysis were also performed using the SAS. Pearson's correlation analyses between the traits were done with the SPSS 16.0 (2009) software.

RESULTS

ANOVA showed that plant height was significantly affected by the urea fertilizer (Table 1). Mean comparison revealed that the application of 160 and 320 kg urea fertilizer increased the plant height by 7% and 15%, respectively, over the control (non-fertilized plants). Also, ANOVA reveled that plant height was noticeably influenced by nano-metal oxide ($P < 0.01$). The tallest plants were recorded under the application of nano-Fe and Zn. However, the interaction effects of urea fertilizer and nano-metal oxide were not significant for this trait. The evaluation of first branch height showed that nano-metal oxide affected this trait at the level of $P = 0.05$. Mean comparison revealed that the application of nano-Cu particles could significantly decrease the height of the first branch. This trait is very important for mechanized harvesting. Our results indicated that fertilizers management can partly affect plants elongation and subjects associated with mechanized harvesting. Urea fertilizer and nano-metal oxide affected the canopy width significantly. The application of 320 kg urea increased lateral growth by 13% over the control. The highest value of canopy width was recorded for the plant grown using nano-Fe and nano-Zn (Table 1). ANOVA showed that the interaction effect of urea fertilizer × nano-metal oxide was statistically significant ($P < 0.01$). The tickets stem was recorded for plant growth under the integrated application of urea and nano-Fe, however, there was no significant difference between the other combined treatments.

Plant dry weight prominently responded to urea fertilizer, so that the application of 160 and 320 kg urea could increase this trait by 24 and 47%, respectively, over control. Mean comparison between the nano-metal oxides showed that plant grown by nano-Fe and Zn had the highest dry weight. The fertilizer management significantly affected the number of secondary branches. The application of 320 kg urea could increase the number of secondary branches by 20% when compared with non-fertilized plants (Table 1). Mean comparison for the number of secondary branches showed that plants grown under the application of nano-Cu had the lowest number of secondary branches.

The number of the total and secondary heads was significantly affected by nano-metal oxides (at 0.05 and 0.01 significance levels, respectively). The plant grown by nano-Fe showed the highest number of heads, followed by the plant grown by nano-Zn. Urea fertilizer only affected the secondary head number ($P < 0.05$). Although the urea application could increase the number of secondary heads, there was no significant difference between 160 and 320 kg urea (U_{160} and U_{320}) applications to plants (Table 1). Mean comparison for head number per plants showed that the highest value was related to plant grown by integrated

Fig. 2. Effects of combined application of urea fertilizer and nano-metal oxide on the number of heads in safflower plants under well irrigated condition;; columns with the same letters are not statistically different; thick horizontal lines in the box represent the average; vertical bars show the standard error; columns with the same letters are not statistically different; thick horizontal lines in the box represent the average; vertical bars show the standard error; LSD = least significant difference

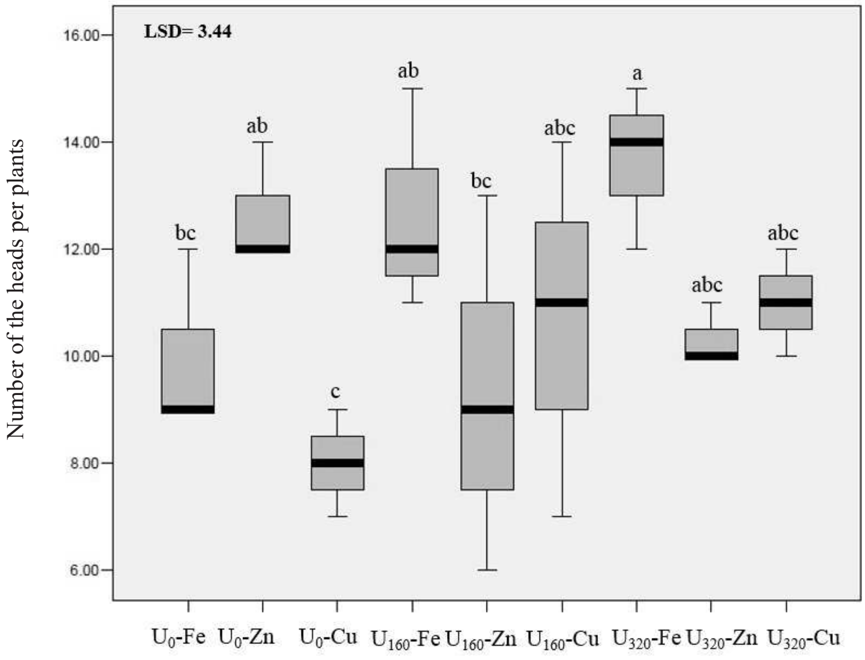

Table 2. Impact of urea fertilizer and metal oxide nanoparticles on yield components of safflower (*Carthamus tinctorius* L.) in Baneh region

	MHD	SHD	SPH	SSH	SNP	TSW	SY	STY	HI
Urea fertilizer	NS	NS	**	*	*	*	**	**	**
non	24.33^a	22.55^a	24.88^c	22.55^a	194.64^b	40.44^a	1259^c	4389^c	22.29^a
160 kg ha^{-1}	25.33^a	21.22^a	35.44^a	21.22^{ab}	234.56^a	37.55^b	1685^b	6217^b	21.37^a
320 kg ha^{-1}	25.60^a	23.22^a	30.33^b	19.00^b	234.00^a	37.77^b	1819^a	7440^a	19.67^b
	nano-metal oxide								
	**	**	*	**	*	*	**	*	NS
Fe_2O_3	27.33^a	23.55^a	33.33^a	22.00^a	250.22^a	38.22^{ab}	1450^{ab}	6104^{ab}	21.44^a
ZnO	25.44^a	23.44^a	31.88^a	23.88^a	239.33^a	40.33^a	1680^a	6292^a	21.05^a
CuO	22.22^b	20.00^b	25.44^b	16.88^b	177.67^b	37.22^b	1230^b	5649^b	20.84^b
U × N	NS	NS	*	**	NS	NS	*	*	NS
CV	10.57	10.04	16.74	13.69	24.89	6.83	7.16	9.29	5.42

MHD = means of main head diameter, SHD = means of diameter in secondary head, SPH = seeds number in main head, SSH = seeds number in secondary head, SNP = number of seeds per plant, TWS = thousand seed weight (g), SY = seed yield (kg ha^{-1}), STW = straw yield (kg ha^{-1}), HI = harvest index (%), U = urea fertilizer, N = micro-nutrient, NS = not significant, CV = coefficient of variance

*significant at 5% level of probability, **significant at 1% level of probability; mean values of the same category followed by different letters are significant at $P \leq 0.05$ level

application of U_{320}-Fe followed by U_{160}-Fe, U_0-Zn (Fig. 2). The lowest number of heads was recorded for plants under the integrated application of U_0-Cu. The assessment of ground cover percentage revealed that this trait was substantially affected by both factors. The highest ground cover belonged to the plant grown by the application of 320 kg urea. Mean comparison of this trait between the nano-metal oxides showed that the application of nano-Fe and nano-Zn increased ground cover percentage by 18 and 11%, respectively, over the nano-Cu (Table 1).

The assessment of unfilled or wrinkled seeds percentage revealed that the highest amount of unfilled seeds was recorded for plants grown by nano-Cu and without the urea fertilizer. The percentage of unfilled seed can significantly affect the final seed yield.

The effects of urea fertilizer and nano-metal oxides on seed yield components are shown in Table 2.

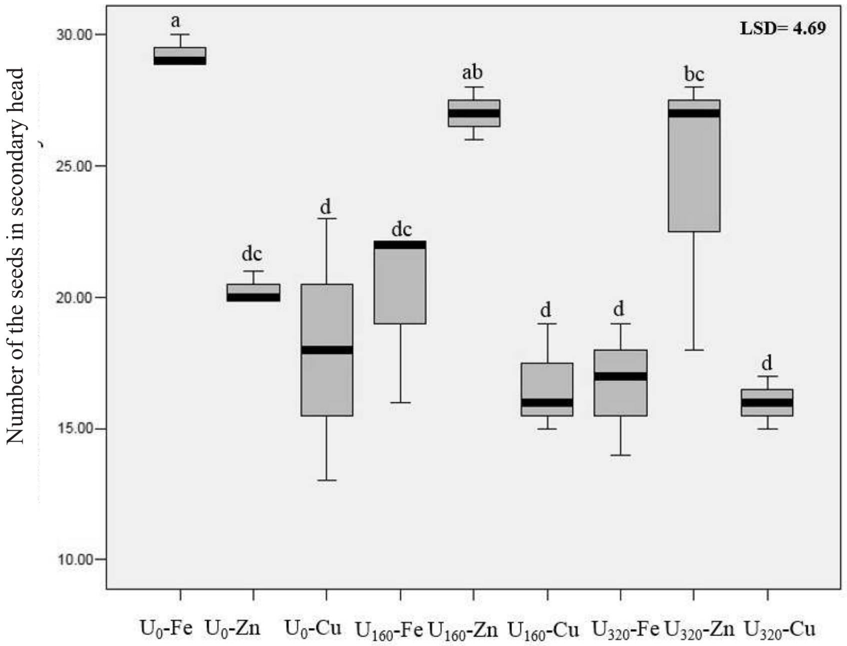

Fig. 3. Number of seeds in the secondary head of safflower affected by combined application of urea fertilizer and nano-metal oxides; columns with the same letters are not statistically different; thick horizontal lines in the box represent the average; vertical bars show the standard error; LSD = least significant difference

ANOVA showed that head diameter was significantly affected only by nano-metal oxide ($P < 0.01$). The largest main head was related to plants grown under the application of nano-Fe and nano-Zn and was by 23 and 14% larger than in plants grown by nano-Cu. A similar trend was observable for secondary head diameter (Table 2). Both factors significantly affected the seeds number per main head, so that the highest seed number was recorded in plants grown by 160 kg urea. Interestingly, the seeds number in plants grown by 320 kg urea was about 15% lower than in plants grown by medium level of urea fertilizer. However, this trend was not recorded for total seeds number per plant. In terms of the seeds number the result of nano-Fe and nano-Zn was similar and the lowest number of seeds per head was related to plant grown by nano-Cu (Table 2).

Mean comparison of seeds number in secondary head between combined treatments revealed that the plants grown using the application of nano-Fe and without urea fertilizer (U_0-Fe) had the highest number of seeds in secondary head, while the lowest amount was recorded for U_{320}-Cu, U_{320}-Fe, and U_{160}-Cu (Fig. 3). TSW was slightly affected by urea fertilizer ($P < 0.05$) and the heaviest seeds were obtained under non-fertilized condition. Application of 160 and 320 kg of urea fertilizer resulted in a 7% decrease in TSW (Table 2). Also nano-metal oxides could affect TSW and the heaviest seed was obtained by the application of nano-Zn.

The assessment of seed yield showed that the main effects of urea fertilizer and nano-metal oxides were significant ($P < 0.01$). Also the interaction effect of urea × nano-metal was statistically significant at the level of 0.05. Mean comparison showed that the highest seed yield was obtained by the integrated application of 320 kg urea and nano-Zn (U_{320}-Zn) followed by U_{320}-Fe and U_{160}-Zn (Fig. 4). The lowest seed yield was recorded in plants grown by no urea along with utilization of nano-Zn and nano-Cu (U_0-Zn and U_0-Cu). The investigation of straw yield showed that the response of this trait to fertilizer managements was mostly similar to seed yield and the highest straw yield was obtained by U_{320}-Zn and U_{320}-Fe.

The vector view of traits and combined treatments is shown by the biplot in Fig. 5. This figure interprets the interrelationship among all measured traits and superiority of the combined treatments. There was a low angel and a positive correlation between seed yield, straw yield, plant dry weight, plant height, number of secondary branches, number of seeds per head, stem diameter, head diameter, and canopy width. The best performances of the mentioned traits were observed by the application of U_{160}-Fe, U_{160}-Zn, and U_{320}-Zn. Also, there was a positive significant correlation between first branch height, first capitulum height, seeds number in secondary head, TSW, harvest index, ground cover percentage, and heads number per plants. These results were also verified by Pearson's correlation analyses (Table 3). The best performance of the formerly mentioned traits was obtained by the integrated application of U_0-Fe and U_0-Zn (Fig. 5). Interestingly, there was a wide angle and a significant negative correlation between percentage of wrinkled

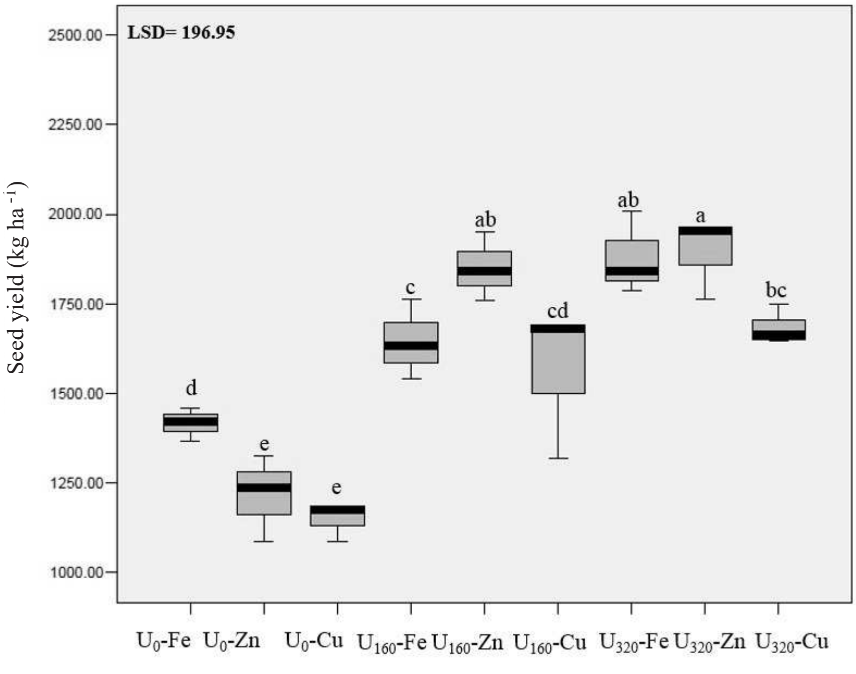

Fig. 4. Effect of integrated application of urea fertilizer and nano-metal oxides on seed yield of safflower in well irrigated condition in Baneh; columns with the same letters are not statistically different; thick horizontal lines in the box represent the average; vertical bars show the standard error; LSD = least significant difference

Table 3. Correlation coefficients between morpho-physiological and agronomic traits of safflower in well irrigated condition

	PH	FBH	FCH	CW	SD	PDW	NSB	NSH	NTH	HNP	MHD	SHD	SPH	SSH	SNP	WSP	TSW	GCP	SY	STY
FBH	0.38																			
FCH	0.15	0.90**																		
CW	0.90**	0.42	0.33																	
SD	0.75**	0.60	0.32	0.67*																
PDW	0.81**	–0.14	–0.30	0.73*	0.41															
NSB	0.89**	0.38	0.23	0.80**	0.51	0.68*														
NSH	0.78**	0.50	0.28	0.75**	0.87**	0.64*	0.51													
NTH	0.68*	0.45	0.29	0.68*	0.77**	0.58	0.33	0.94**												
HNP	–0.56*	0.04	0.14	–0.45	–0.40	–0.60*	–0.67*	–0.39	–0.14											
MHD	0.81**	0.74*	0.64*	0.87**	0.73*	0.47	0.81**	0.75**	0.61	–0.50										
SHD	0.60*	0.44	0.46	0.61*	0.14	0.34	0.81**	0.14	0.10	–0.28	0.64*									
SPH	0.59*	0.26	0.17	0.58*	0.38	0.48	0.74*	0.40	0.15	–0.63*	0.68*	0.38								
SSH	0.12	0.36	0.35	0.05	–0.21	–0.15	0.42	–0.31	–0.35	0.16	0.21	0.67*	0.33							
SNP	0.89**	0.49	0.28	0.89**	0.72*	0.68*	0.85**	0.76**	0.57	–0.56*	0.89**	0.51	0.81**	0.17						
WSP	–0.91**	–0.25	0.00	–0.81**	–0.61*	–0.86**	–0.87**	–0.73*	–0.55	0.69*	–0.75**	–0.48	–0.76**	–0.11	–0.93					
TSW	–0.14	0.27	0.45	0.04	–0.42	–0.35	0.12	–0.47	–0.40	0.46	0.06	0.58*	0.01	0.80**	–0.05	0.22				
GCP	–0.44	0.35	0.52	–0.23	–0.24	–0.66*	–0.48	–0.30	–0.08	0.90**	–0.18	–0.02	–0.47	0.29	–0.38	0.63*	0.64*			
SY	0.66*	0.25	0.30	0.76**	0.55*	0.71*	0.62*	0.65*	0.57*	0.71*	0.73*	0.82**	0.44	–0.76**	0.80**	–0.59*	–0.69*	–0.76**		
STY	0.67*	0.21	0.25	0.76**	0.54	0.64*	0.61*	0.65*	0.58*	–0.72*	0.71*	0.41	0.42	–0.32	0.59*	–0.60*	–0.31	–0.49	1.00**	
HI	–0.38	0.55*	0.71*	–0.24	–0.13	–0.75*	–0.18	–0.31	–0.31	0.42	0.10	0.08	0.08	0.49	–0.15	0.45	0.59*	0.68*	–0.27	–0.33

PH = plant height at maturity, FBH = first branch height, FCH = first capitulum height, CW = canopy width, SD = stem diameter, PDW = plant dry weight, NSB = number of secondary branches, NSH = number of heads in secondary branches, NTH = total number of heads in plants, WSP = percentage of wrinkled seeds, GCP = ground cover percentage, MHD = means of main head diameter, SHD = means of diameter in secondary head, SPH = seeds number in main head, SSH = seeds number in secondary head, SNP = number of seeds per plant, TSW = thousand seed weight, SY = seed yield, STY = straw yield, HI = harvest index

*, **significant at the 0.05 and 0.01 probability levels, respectively seeds and seed yield ($r = \cos 180° = -1$). The highest amount of unfilled seeds was recorded for plants grown by the integrated application of U_0-Cu.

DISCUSSION

Our results revealed that morphological characteristics were affected by the urea fertilizers. Nitrogen deficiency is one of the most important limiting factors in semi-arid regions. This finding is supported by previous research showing that nitrogen application could increase plant growth and seed yield by modification of many physiological processes (Shangguan et al., 2004). With regards to the critical role of nitrogen in photosynthetic apparatus such an increase in growth parameters was predictable. Leaf nitrogen is mostly used for the synthesis of components of the photosynthetic apparatus, and about 75% of leaf nitrogen is allocated to the chloroplasts (Shangguan et al., 2000). The positive effect of nitrogen application in this study is in agreement with the finding of Yang et al. (2006) who showed that a long term application of nitrogen fertilizers enhanced crop productivity and improved nutrient cycling within soil. However the best result was obtained by the combined application of P, K, and farmyard manure.

Although the main effects of nitrogen in plants have already been studied previously, our study was novel due to the evaluation of the combined effect of nitrogen and micronutrients nanoparticles on safflower in cold semi-arid areas. The previous research sufficiently explained the positive effects of nitrogen as it is the main part of amino acids and is crucial for protein biosynthesis. However our result revealed that the efficiency of nitrogen can be affected by the availability of micronutrients and *vice versa*. This experiment is among the few studies focused on the interaction of nitrogen with nano-scale micronutrients.

Our findings showed that the integrated application of U_{160}-Fe, U_{160}-Zn, and U_{320}-Zn significantly improved the morphological traits. Our results are in line with some previous reports in this field (e.g. Galavi et al., 2012). It is encouraging to compare our results with those by Gul et al. (2011) who found that two spray applications of 0.5% N, 0.5% K, and 0.5% Zn solutions resulted in the highest number of tillers, spikes, and the greatest plant height in wheat plants. In plants, zinc is a key constituent of many enzymes and proteins. It plays an important role in a wide range of processes, such as growth hormone production and cell elongation. Zinc plays critical roles in many enzymes, often appearing either at the active site of the enzyme or in a position that regulates the enzyme structure. Plants deficient in zinc often show symptoms known as little leaf and rosette growth (Marschner, 2012). In the case of little leaf, the leaves fail to expand to their normal mature size and this can dramatically affect the sink–source relationship and finally decrease the seed yield. Rosette plants are those in which elongation of the stem is almost eliminated, so that all leaves appear to grow from the same place at the base of the stem. Zinc deficiency can also result in stunted growth forms (Wiedenhoeft, 2006).

Our findings revealed that the lowest plant growth performance was recorded under the copper application condition. Copper can interact with organic matter functional groups such as carboxyl and phenol to form inner sphere complexes and these may affect plant available pool and environmental fate. Copper absorption in plants may be affected also by the availability of other nutrients, e.g. it has been shown to be limited by elevated zinc, iron, and phosphorus concentrations in soil. The copper fixation pattern in semi-arid soils

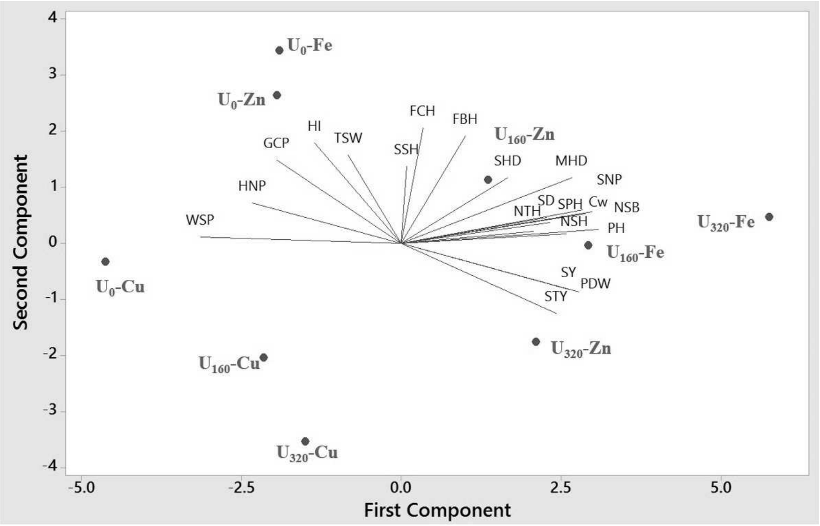

Fig. 5. Vector view of combined treatments by trait biplot, showing the interrelationship among all measured traits and superiority of the combined treatments. Low angles between lines show the high positive correlation; PH = plant height at maturity, FBH = first branch height, CW = canopy width, SD = stem diameter, PDW = plant dry weight, NSB = number of secondary branches, NSH = number of heads in secondary branches, NTH = Total number of heads in plants, WSP = percentage of wrinkled seeds, GCP = ground cover percentage, MHD = means of main head diameter, SHD = means of diameter in secondary head, SPH = seeds number in main head, SSH = seeds number in secondary head, SNP = number of seeds per plant, TWS = thousand seed weight, SY = seed yield, STW = straw yield, HI = harvest index

was influenced by pH and the combination of pH and calcium carbonate (Udeigwe et al., 2016).

A concomitant application of micronutrients and a high level of nitrogen increased the lateral growth and stem elongation significantly. This increase could be due to important roles of micronutrients in the biosynthesis of phytohormones. It has been revealed that Zn plays an important role in regulating the auxin concentration in plants and it is an essential component of enzymes which promote the growth and development of plants. It is worth noting that growth and development of plants are regulated by phytohormones; our finding showed that growth characteristics were influenced by the application of micronutrients (especially Zn and Fe) and nitrogen. These results are in line with the findings of Arora, Singh (2004) who reported that the combined application of 7.5 kg Zn along with 90 kg N ha^{-1} resulted in the maximum grain yield and straw yield.

Nitrogen fertilization can promote Fe and Zn accumulation in grains. It seems that even the type of nitrogen fertilizer also affects the ratio of adsorption and bioavailability of micronutrients (Barunawati et al., 2013). It has been reported that, compared to nitrate, the supply of ammonium to hydroponically-grown wheat plants resulted in a general increase in the concentrations of many nutrients, including Fe, Cu as well as Zn, and particularly bioavailable Zn (Carlisle et al., 2012). Because the soils of semi-arid area are often calcareous, the application of cationic form of nitrogen can be more beneficial.

Cu is involved in several enzyme systems, cell wall formation, electron transport, and oxidation reactions. Deficiency of Cu results in slow growth, spotted and deformed leaves (Marschner, 2012). Despite this fact, the lowest growth performance was observed in plant grown by the application of nano-Cu. The efficiency of cooper is strongly affected by soil condition, plants types, and availability of other nutrients (Monreal et al., 2015). There are, however, several reports indicating no improvement or toxicity from Cu fertilizers (Shi et al., 2011; Atha et al., 2012; Wang et al., 2012). In general, it seems that Cu deficiency usually occurs in irregular patches within fields and the utilization of copper fertilizers should be vigilant and precise. On the other hand, due to their small size and large surface, nano-particles are prone to aggregation in aqueous phase, which may influence their bioavailability and toxicity (Lin, Xing, 2008). However, our result revealed that unlike the individual use, the integrated application of nitrogen and Cu could improve growth and seed yield. Nevertheless, nano-structured formulation through instruments such as targeted delivery or slow/controlled release mechanisms and conditional release, could release their active ingredients in responding to environmental triggers and biological demands more precisely (Naderi, Danesh-Shahraki, 2013). To achieve environmental stability and sustainable productivity through rational nutrient management is becoming an important issue in the developing ecosystem of semi-arid regions. This study produced results which corroborate with findings of a great deal of previous work in this field. Rong et al. (2016) in a 7-year field experiment in newly cultivated farmland of semi-arid region revealed that the integrated application of chemicals and farmyard manure can yield significant benefits for soil C and N accumulation and improve productivity of ecosystem. Our findings showed that the application of micronutrients fertilizers in safflower can substantially increase seed yield as well as nitrogen use efficiency and a positive (synergistic) interaction effect on crop growth and yield was observed. The findings of the current study are consistent with those of Fageria (2001). However, these nutrient interactions in plants can be influenced by several factors, are very complicated and much remains to be learned about their specific mode of action.

CONCLUSION

The study demonstrated a significant interaction between nitrogen and micronutrients. Results indicated a high positive correlation between seed yield and canopy width, straw yield, seeds number per plant, number of secondary heads, plant height, and head diameter. The best performance was recorded for plant grown by the integrated application of U_{160}-Fe and U_{320}-Zn. The lowest performance of seed yield was recorded for plants grown by U_0-Cu. Overall, the current study highlighted the effectiveness of the use of nano-structured micronutrient fertilizer along with nitrogen fertilizer. This result refers to the importance of micronutrients applicationin semi-arid regions.

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Corresponding Author:

Mohsen Janmohammadi (PhD), University of Maragheh, Faculty of Agriculture,Department of Plant Production and Genetic, Maragheh, P.O. Box 55181-83111, Iran, phone: +98 41 3727 3070, e-mail: jmohamad@alumni.ut.ac.ir