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The effect of concomitant use of nano-structured essential metals and sulfur on growth characteristics of safflower

ABSTRACT

Deficiencies of zinc, manganese and iron are common in calcareous soils of arid and semiarid regions due to their reduced solubility alkaline conditions. However, sustainable crop production is essential for growing population. Sulfur fertilizers can increase micronutrients availability by decreasing soil pH. In order to investigate the influence of nano-chelated essential metals (Zn, Mn, Fe) and sulfur application (zero and 40 kg ha^{-1}) an experiment was carried out in Maragheh, northwest of Iran. Phenological development, morphological and agronomic traits significantly responded to both factors. Results revealed that application of sulfur fertilizer considerably increased morphological traits such as ground cover, stem diameter, plant height and capitulum diameter. Mean comparison between nano-chelated metal showed that the highest value for seed yield and yield components (number of the capitulum per plants, seed number per capitulum and seed weight) was achieved through the application of nano-chelated Zn. The best performance was related to combined application of sulfur and nano-chelated Zn which was followed by nano-chelated Fe. Seed oil content was only affected by nano-metals, so the highest value was obtained by application of nano-chelated Zn. Overall our finding revealed that integrated application of sulfur and essential metals, especially Zn, is required to grow safflower successfully on calcareous soils. The efficiency of nano-chelated fertilizers can be noticeably increased by balanced nutrient management in semi-arid regions.

Keywords: balanced nutrition, calcareous soils, combined application, nano-chelated micronutrients, nano zinc oxide

STRESZCZENIE

Niedobory cynku, manganu i żelaza są powszechne w wapiennych glebach suchych i regionach półpustynnych z powodu czynników alkalicznych, ograniczających ich rozpuszczalność. Jednak zrównoważona produkcja roślinna jest konieczna dla rosnącej populacji ludności. Nawozy siarkowe mogą zwiększyć dostępność mikroelementów przez obniżenie pH gleby. W celu zbadania wpływu nano-chelatowanych mikroelementów (Zn, Mn, Fe) i siarki (0 i 40 kg ha^{-1}) przeprowadzono eksperyment w Maragheh, na północnym zachodzie Iranu. Rozwój fenologiczny, cechy morfologiczne i agronomiczne zależały od zastosowanych pierwiastków. Wyniki wykazały, że stosowanie nawozów siarkowych znacznie poprawia cechy morfologiczne, takie jak pokrycie gruntu, średnicę łodygi, wysokość pędu i średnicę kwiatostanu. Porównanie zastosowanych form nano-chelatowanych metali wykazało, że najwyższą wartość plonu nasion i innych parametrów (liczba kwiatostanów na roślinę, liczba nasion w główce i masa nasion) osiągnięto po zastosowaniu nano-chelatowanego Zn. Najlepszy wzrost roślin był uzyskany po jednoczesnym zastosowaniu siarki i nano-chelatowanego Zn, a w mniejszym stopniu po zastosowaniu Fe. Najwyższą zawartość oleju w nasionach uzyskano poprzez zastosowanie nano-chelatowanego Zn. Podsumowując, uzyskane wyniki wykazały, że zintegrowana aplikacja siarki i metali, zwłaszcza Zn, są niezbędne do uprawy krokosza na glebach wapiennych. Efektywność działania nano-chelatowanych nawozów zauważalnie wzrosła przy zrównoważonym stosowaniu składników mineralnych w regionach półpustynnych.

Słowa kluczowe: zrównoważone nawożenie, gleby wapienne, aplikacja równoczesna, nano-chelatowane pierwiastki śladowe, nano tlenek cynku

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf and highly branched crop from family Asteraceae and is suited to lower rainfall and lower input farming (35). This plant is an important oilseed crop that originated from eastern Mediterranean regions. The estimated world production is about 0.647 million tons of seed per year from about 0.783 million ha (5). The largest cultivated area of safflower is related to Kazakhstan, China, India and the United States, while leading producers are the United States, Mexico, Australia, China, Argentina and Kazakhstan (5). The highest seed yield is related to China (1565 kg), Turkey (1536 kg) and the United States (1381 kg). Additionally, after oil extraction, the safflower meal is used for ruminant feed, and can be used for poultry feed if safflower seed is de-hulled before pressing. Safflower, due to extensive root system with a strong fleshy taproot is tolerant against water deficit condition (37). Also, it is a valuable forage for Mediterranean areas since it remains green and has a higher feed value under dry conditions. Safflower is a valuable forage provided it is harvested from mid-budding to early blooming stage (16). Safflower has a deep root system allowing the plant to utilize efficiently the moisture and nutrients that may not be available to small-grain crops (8).

Although safflower is considered a minor crop with less than 1 million hectares planted, producing considerable amount of seed each year, it plays an important role within the farming systems (8). Beside the relatively higher price of safflower seed in the world market, growing safflower is estimated to give much higher economic returns than barley (36). Nonetheless, it has mainly remained a minor crop grown on small plots for the growers' personal use. It seems that water and nutrients are the main limiting factors in the majority of Mediterranean semi-arid ecosystems (2).

However, in semi-arid regions, the loss of organic matter and low fertility are of great concerns. Low soil organic matter, is related to the historically low level of plant production and high rates of organic-matter decomposition. Soil organic matter controls soil nutrients that affect biomass and contributes to soil fertility by serving as a source of plant nutrients (32). Likewise, calcareous soils cover more parts of semi-arid regions and their $CaCO_3$ content varies from a few percent to 95%. This problem occurred because of low precipitation and relatively little leaching (17). Micronutrient deficiency of plants occur more frequently in calcareous soils with high pH such as those found in semi-arid regions (1). Moreover, although Green Revolution increased crop production per unit area, it also has resulted in greater depletion of available micronutrients in soils. After this development the most of concerns focused on high yield production through macronutrients application as less attention has been paid to micronutrients fertilization (14). Therefore, nutrient deficiencies or nutrient imbalance is one of the main problems in semi-arid environment and leads to restricted crop production (31). Despite the mentioned barriers, soils of these regions can be extremely productive for agricultural use when they are managed properly.

Nutrient management and soil improvement play an important role in the universal necessity to increase crop production and meet the food needs of the growing population. Zinc, iron and manganese deficiencies are recognized as the widest spread plant nutrient disorders in semi-arid region (28). However, it appears that utilization of acidifying materials such as elemental sulfur could be a possible and economic way for decreasing soil pH and improving the availability of micronutrients in calcareous and alkali soils (10). It has been revealed that by application of sulfur fertilizer in soil, oxidizing bacteria particularly *Thiobacillus* spp. would accelerate the oxidation process and convert the sulfur to sulfuric acid which leads to soil pH decline (22). In addition, sulfur serves many functions in plants and it is necessary for formation of amino acids, proteins, oils and chlorophyll (4). Sulfur also has some crucial roles in activation of certain enzymes and vitamins (20).

Fertilizers have fundamental role in improving the crop production particularly after the introduction of high yielding and fertilizer responsive varieties. However, the efficiency of conventional fertilizers is relatively low. Accordingly, in recent years some modern fertilizers have been introduced and among them the use of nano based fertilizers is growing (24). Nano-fertilizer is formulated by nano-structured nutrients and applied nanoparticles have large surface area to the volume ratio, which provides better opportunity for interaction. Due to the larger surface area in nanoparticles, they are estimated to be more biologically active than conventional fertilizers with bulk particles of the same chemical composition (30). Nano based fertilizers are known to release active nutrients gradually and steadily during the months which may assist in improving the nutrient use efficiency without any deficiency symptoms (33). In some nano-fertilizers nutrients are released in response to environmental signal like fluctuations in soil moisture or temperature, thus this smart delivery of active ingredients can significantly minimize the nutrient losses in fertilization (26). Indeed, utilization of modern fertilizers applying innovative nanotechnology is one of the potentially operative options of considerably increasing the global agricultural productions required to meet the forthcoming demands of the growing population (19). Although some sporadic studies elevated the effects of nano-structured zinc, iron and manganese on plants growth (7, 13, 29, 38), there is not sufficient information about interaction of sulfur with nano-micronutrients in calcareous soils of semi-arid region. Thus, this study aimed to determine the effect of integrated application of nano-structured essential metals and sulfur on growth and yield components of safflower under semi-arid highlands conditions.

MATERIALS AND METHODS

The experiment was conducted at the Research Field of the University of Maragheh. The district (46° 16’ E and 37° 23’ N) is located at 1,485 m above sea level in the semi-arid eastern Azarbajian, northwest of Iran. The area has a Mediterranean-type of climate. According to the updated classification of Köppen and Geiger, its climate is classified as BSk; cold semi-arid climate (27). The average long-term annual precipitation is 353 mm, 71% of which falls in November, December, January and February. Average maximum and minimum temperature during growing season was 25°C and 13°C, respectively. The climatic conditions of the trial are depicted in Table 1, indicating monthly rainfall and temperatures during growing season. The experiment was established on a silty loam soil (25% clay, 51% silt and 24% sand) with pH 8.07, organic matter content 0.92%, total nitrogen 0.17%, $Caco_3$ 17%, electrical conductivity (*EC*) 0.8 ds m^{-1}, phosphorus 15.31 ppm and potassium 820 ppm (at 0–40 cm depth before fertilizer application). The previous crop in the experimental field was lentil (*Lens culinaris*).

Table 1. Meteorological parameters during crop seasons of 2016 at Maragheh station

Climatic parameters	March	April	May	June	July	August
Precipitation (mm)	43	51	2057	25.9	13	0
Mean humidity (%)	57	50.2	40.1	31.7	30.8	21
Total evaporation (mm)	14	32	49	193	278	335
Mean temperature (°C)	8.5	13.7	19.2	23.9	26.3	29.6

The facultative safflower cultivar "Goldasht", which is widely adapted to temperate-cold region, was used in experiments. Early maturity, higher number of heads per plant, head size, spinelessness are the main characteristics of this new cultivar. Early maturity (20–25 days earlier than commercially grown cultivars) is one the most important characteristics of this cultivar. The area was mouldboard-ploughed and disked before planting. The experimental design was a split plot in randomized complete block design with three replications. Main plot treatments consisted of two elemental-sulfur fertilizer rates: 0 and 40 kg S ha^{-1}. Subplot treatments were four nano-chelated metal fertilizer control (non-application), nano-chelated zinc (ZnO), nano-chelated manganese (MnO) and nano-chelated iron (Fe_2O_3). Elemental S was spread over the soil surface by hand before the sowing of the crop, and was incorporated into the top 10 cm of soil using rotary hoe. Nano-chelated fertilizers were obtained from the Sepeher Parmis Company, Iran, which contained zinc oxide, ferric oxide and manganese (II) oxide nanoparticles. Synthesized nanoparticles had been characterized morphologically by transmission electron microscope (Fig. 1). Nano-chelated fertilizers were applied at rate of 1 kg ha^{-1}. The first half of the nano-fertilizer was broadcast

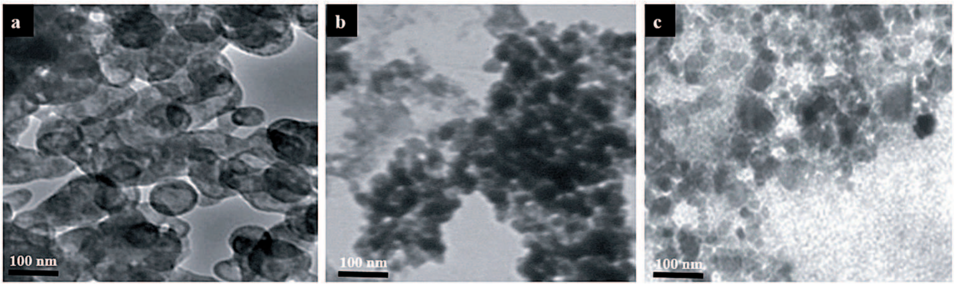

Figure 1. Transmission electron microscopy (TEM) micrograph of ferric oxide (a), manganese oxide (b) and zinc oxide (c) nanoparticles utilized for production of nano-fertilizers.

by hand and incorporated immediately before planting. The subsequent part of nano-fertilizers was applied 60 days after sowing (BBCH scale= 50; capitulum emergence) as fertigation through irrigation water. Plant growth stages and phonological development were determined according to methods of Flemmer et al., (2015). Seeds were hand planted on 27 April in eight-row plots, 2 m long with a spacing of 0.4 m between rows, and at a rate of 20 seeds per meter of row. Filed was irrigated immediately after planting and thinned at the rosette stage. Small terraces of 1.5 m were considered in the interspaces to prevent contamination by surface run-off containing fertilizer. There was no incidence of pest or disease on plants during the experiment. The crop was kept free of weeds by hand hoeing when necessary. The trial plots were irrigated six times at intervals of seven to ten days, using a furrow irrigation system. Phenological growth phase were monitored at 1–2 day intervals throughout the season. A portable chlorophyll meter (SPAD-502) was used to measure the amount of chlorophyll in fully expanded leaves at flowering stage (BBCH= 50, beginning of capitulum formation, still enclosed by leaves). Groundcover was determined during the flowering stage (BBCH= 65; 50% of florets open in flowers on main shoot) as amount of plant material (dead or alive) that covers the soil surface. It was expressed as a percentage through visual assessment; 100% groundcover means that the soil cannot be seen and 0% groundcover is bare soil.

Plants were hand-harvested at physiological maturity (30 August).Yield components were recorded from 10 randomly selected plants in both years. The biological yield, plant height, capitulum diameter, percentage of unfilled seeds, number of capitulum per plant, seed yield, 1,000-seed weight and harvest index were evaluated after harvesting. Seed yield in t ha^{-1} was obtained by harvesting the four central rows of the plot by hand. The oil content was analyzed by the Soxhlet oil extraction technique according to the described method by Mohsennia and Jalilian (2012). Analysis of variances of data (ANOVA) for each attribute and combined analysis of the split plot designs were computed using the SAS computer program. The MSTATC software package was used to test significant interaction effects between treatments. Differences in character means were also measured using the Least Significant Difference (LSD). Correlation analysis and principal component analysis (PCA), based on the rank correlation matrix and biplot analysis were performed by SPSS ver. 16, STATISTICA ver. 8 and Minitab ver. 16.

RESULTS

Results of variance analysis for morphological and phenological traits are depicted in Table 2. The main effect of sulfur and nano-structured metal on ground cover percentage was significant ($P < 0.05$), also the interaction effects of sulfur × nano-metal was statistically significant. The highest ground cover was recorded for plant grown by application of sulfur and nano-Zn (85%) and the lowest amount (55%) was related to plants grown under control condition (non-application of sulfur and nano-metal). Plant height was noticeably affected by both factors, sulfur application increased this trait up to 19% over control. Mean comparison between nano-metals revealed that the tallest plants were obtained by application on nano-Zn. A similar trend was observed for stem diameter, so that sulfur utilization increased stem diameter up to 40% over control, and between the levels of nano-structured metals the thickest stem was related to plant grown by nano-Zn and Fe.

Evaluation of chlorophyll content showed that sulfur application significantly enhanced the amount of this pigment ($P < 0.01$; up to 16%). Likewise, the effect of the nano-metal on chlorophyll content was significant ($P < 0.01$), so that the highest value was recorded for plant grown by nano-Fe. The impact of nano-Fe on chlorophyll content was very remarkable compared to other nano-metal fertilizers

Table 2. Effect of sulfur and nano-structured metals on some morphological traits of safflower (*Carthamus tinctorius* L.)

Sulfur	GC	PH	SD	CHL	DCE	DF	DM	CD	BY
	*	**	**	**	**	**	**	**	**
Non	67.33b	59.00b	4.13b	44.33b	56.50a	74.25a	110.75b	2.77b	3912.96b
With	75.91a	69.91a	5.85a	51.41a	51.50b	67.25b	116.50a	3.31a	4231.00a
	Nano-structured metals								
	*	**	*	**	*	**	**	*	**
Control	61.66c	58.83c	4.47b	42.33c	58.83a	74.83a	107.50c	2.42c	3747.31c
Nano-Zn	80.83a	70.50.a	5.38a	46.00b	48.66c	65.66c	118.50a	3.44a	4285.90a
Nano-Mn	69.33b	63.00bc	4.58b	43.00c	56.66a	72.50ab	113.83b	3.03b	4056.41b
Nano-Fe	74.66ab	65.50b	5.53a	60.16a	51.83b	70.00b	114.66b	3.28ab	4198.8ab
S×M	*	NS	NS	NS	*	*	NS	NS	NS
CV%	7.68	5.56	11.44	4.89	4.53	3.84	1.92	10.53	4.39

GC – ground cover (%), PH – plant height at maturity (cm), SD – stem diameter (mm), chlorophyll content (SPAD unit), DCE – number of days to capitulum emergence, DF – days to initiation of flowering, DM – days to maturity, BY – biological yield (kg ha^{-1}), CD – capitulum diameter. S – sulfur, M – nano-structured metals, CV – coefficient of variance, NS – not significant, * – 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≤0.05 level.

(Table 2). Phenological development significantly responded to sulfur fertilizer, so that application of sulfur considerably accelerated the capitulum emergence and flowering while significantly delayed physiological maturity. The effect of nano-metals on mentioned trait was also noticeable and the earliest capitulum emergence and flowering was recorded for plants grown by nano-Zn. However, nano-metals considerably prolonged the reproductive growth and the longest period of growth (days to maturity) was recorded for plants grown by nano-Zn. The interaction effects of sulfur × nano-metal statistically was significant for the number of days to capitulum emergence and the number of days to initiation of flowering (Table 2). Mean comparison showed that plant grown by integrated application of sulfur and nano-Zn initiate the reproductive stage much earlier than other treatments (Fig. 2). Capitulum diameter, as an important trait, was significantly increased by sulfur application, up to 20% over control. On the other hand, mean comparison between nano-metals revealed that the biggest capitulum was obtained by application of Zn and Fe. However, the nano-manganese application increased the capitulum diameter up to 25% over the control (Table 2). Evaluation of biological yield revealed that sulfur application increased this parameter up to 9% over the control. Also, nano-metal considerably improved biological yield and the most prominent effect was related to Zn and Fe, respectively.

The effects of sulfur and nano-metals on yield components and oil content are illustrated in Table 3. The highest number of the capitula in primary branch

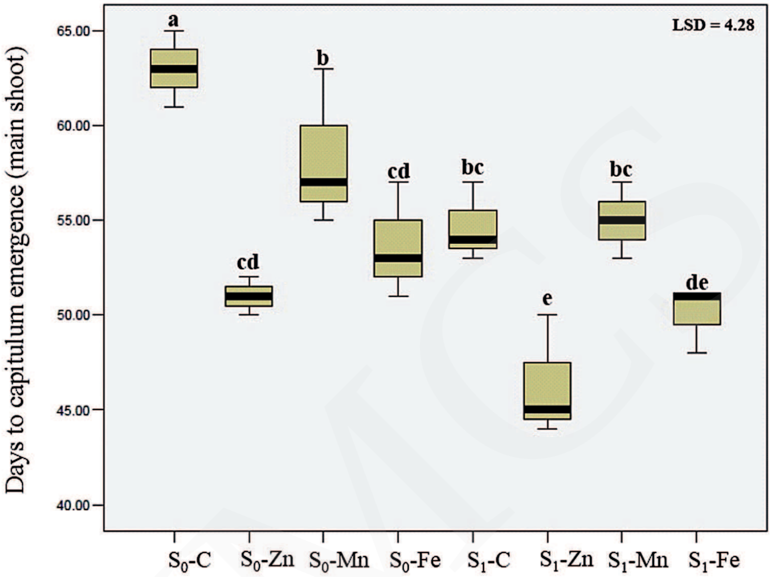

Figure 2. Effects of integrated application of sulfur and nano-metals on the number of days to capitulum emergence in safflower grown in semi-arid region of Maragheh. C – non-application of nano-metals, S_0 – without sulfur application, S_1 – application of 40 kg ha^{-1} elemental sulfur, LSD – least significant difference. Means followed by a common letter are not significantly different at the 5% level. Vertical bars show standard error.

Table 3. Yield and yield components of safflower (*Carthamus tinctorius* L.) affected by sulfur and nano-structured metals

Sulfur	NCM	NCS	CPP	SPC	TSW	USP	SY	HI	OIL
	**	**	**	**	NS	**	**	NS	NS
Non	3.00b	4.62b	9.09b	21.66b	29.84a	6.02a	1215.09b	30.98a	23.64a
With	4.12a	6.00a	12.23a	26.58a	30.53a	4.42b	1299.53a	30.74a	24.15a
	Nano-structured metals								
	**	**	*	**	**	**	**	*	*
Control	2.33c	3.58c	7.42c	18.00c	28.27c	7.36a	1083.07c	28.58b	22.15b
Nano-Zn	4.58a	7.46a	14.46a	26.83ab	31.72a	4.21c	1359.83a	31.82a	26.12a
Nano-Mn	3.33b	5.28b	10.34b	23.16b	30.12b	5.30b	1257.00b	31.04a	23.00b
Nano-Fe	4.00a	4.64bc	10.45b	28.50a	30.61ab	4.01c	1329.17a	31.74a	24.30ab
S×M	NS	**	**	*	NS	*	*	*	NS
CV%	7.59	17.84	10.05	14.21	3.15	16.07	3.67	4.72	8.73

NCM – number of capitula in main branch, NCS – number of capitula in secondary branches, CPP – number of capitulum per plant, SPC – seed number per capitulum, TSW – thousand seeds weight (g), USP – unfilled seed percentage, SY – seed yield (kg ha^{-1}), HI – harvest index, OIL – oil content (%), S – sulfur, M – nano-structured metals. CV – coefficient of variance. NS – not significant, * – 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≤0.05 level.

was obtained by Zn and Fe fertilizers. Also the application of sulfur increased this yield parameter up to 37% over the control. The interaction effects of sulfur × nano-metal on the number of the capitula in secondary branches was statistically significant and the greatest number was obtained by integrated application of nano-Zn and sulfur fertilizer. A similar pattern was observed for a number of the capitula per plants and best performance was recorded for plant grown by concomitant application of sulfur with Zn, Fe and Mn, respectively (Fig. 3). Also the superiority of nano-Zn under sulfur free condition was prominent. Assessment of seed number per capitulum revealed that integrated application of sulfur and nano-Zn or Fe produced the highest seed number. However, under non-sulfur application the highest number was obtained by nano-Fe fertilizer (Fig. 4). Evaluation of seed weight showed that only nano-metal affected this trait. So that application of nano-Zn, Mn and Fe increased seed weight up to 12%, 7% and 9%, respectively. Fertilizer application noticeably decreased the percentage of unfilled and shriveled seeds. The highest proportion of unfilled seeds was recorded under control condition (no-fertilizer application) and was followed by plant grown under sulfur application without nano-metal utilization. The lowest proportion of unfilled seeds was recorded under concomitant application of sulfur and nano-Zn (Fig. 5). Seed yield was also affected by both factors, the highest yield was obtained by

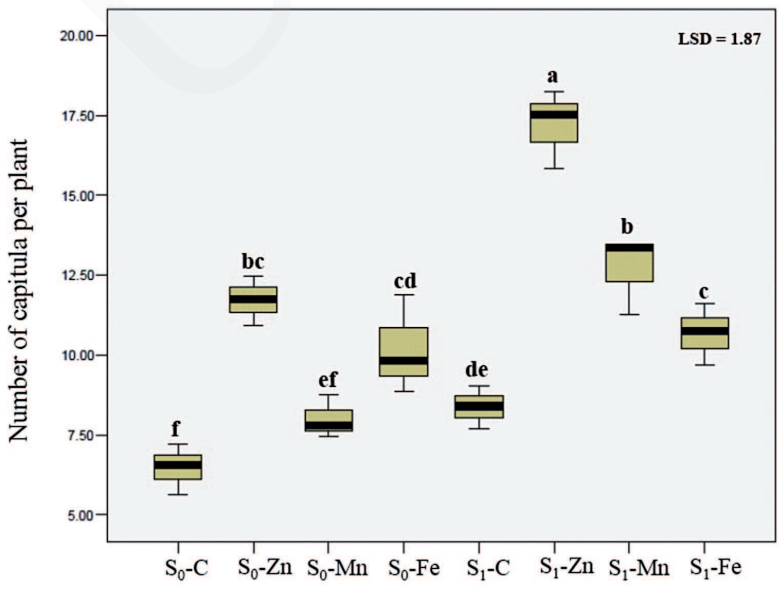

Figure 3. Impact of nano-metals and sulfur fertilizer on the number of capitula in safflower plants, in highland semi-arid region of Maragheh. C – non-application of nano-metals, S_0 – without sulfur application, S_1 – application of 40 kg ha^{-1} elemental sulfur.

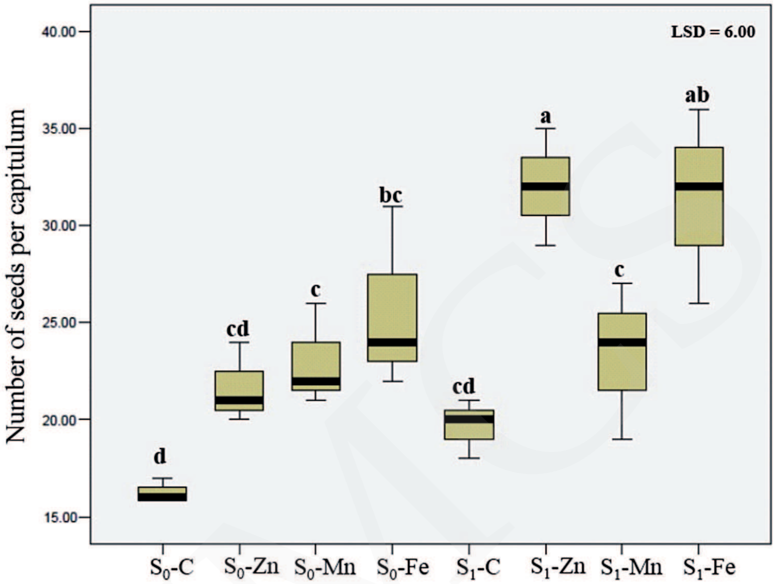

Figure 4. Number of the seeds per capitulum in safflower plants as affected by integrated application of nano-metal and sulfur fertilizers. C – non-application of nano-metals, S_0 – without sulfur application, S_1– application of 40 kg ha^{-1} elemental sulfur. Means followed by a common letter are not significantly different at the 5% level.

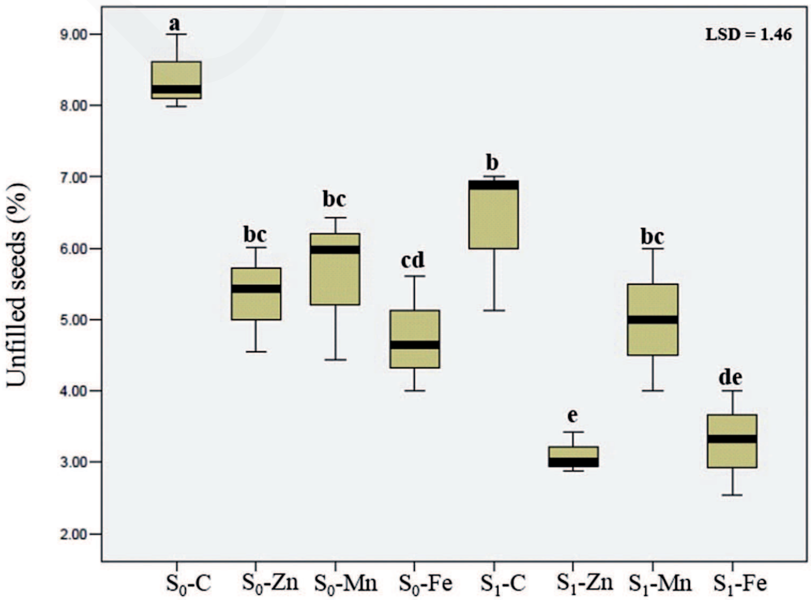

Figure 5. Effect of sulfur and nano-metal fertilizers on percentage of unfilled and shriveled seeds in safflower plants grown in highland semi-arid region of Maragheh. C – non-application of nano-metals, S_0 – without sulfur application, S_1: application of 40 kg ha^{-1} elemental sulfur.

integrated application of sulfur and nano-Zn (Fig. 6). Although under sulfur free condition seed yield was significantly lower than sulfur applied condition, nano-Zn application substantially improved seed yield under sulfur free condition.

Even though the main effect of sulfur was statistically insignificant on harvest index, the interaction effect of sulfur × nano-metal was significant. Mean comparison showed that combined application of sulfur and nano-metals can lead to the highest values of harvest index. Investigation of oil content showed that only the effect of nano-metals was significant and the application of zinc and iron led to the highest oil content.

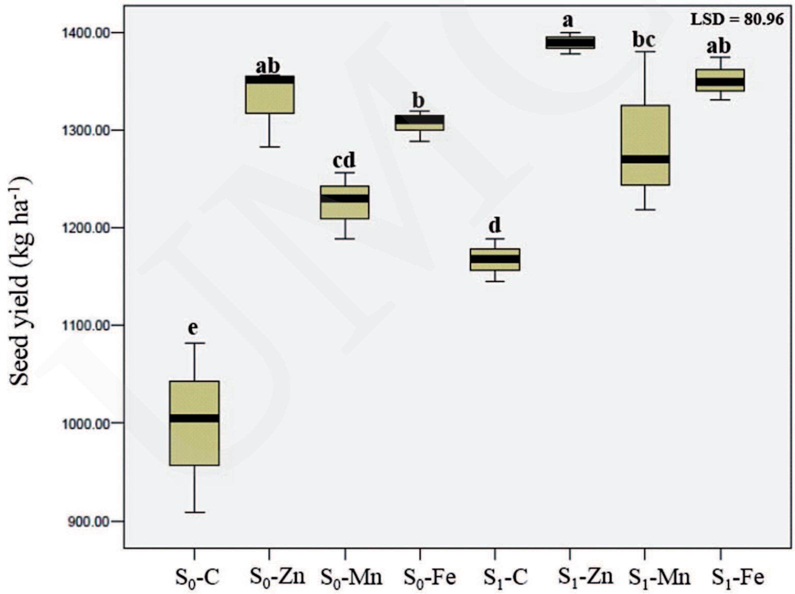

Figure 6. Seed yield of safflower as affected by concomitant application of sulfur and nano-metal fertilizer. C – non-application of nano-metals, S_0 – without sulfur application, S_1 – application of 40 kg ha^{-1} elemental sulfur. Means followed by a common letter are not significantly different at the 5% level.

Cluster analysis was used to classify the effects of the treatments on evaluated traits. Cluster analysis of treatments showed that the dendrogram was divided into three groups (Fig. 7). Group I consisted of S_0-C (no fertilizer application), which showed the lowest vegetative growth and seed yield. These results indicate the importance of the sulfur and micronutrients in the studied area. Group II contained the S_0-Zn (non-sulfur application with nano-Zn utilization), S_0-Mn (non-sulfur application with nano-Mn consumption) and S_0-Fe (non-sulfur application with nano-Fe use). This result refers to this important note that application of nano-metals

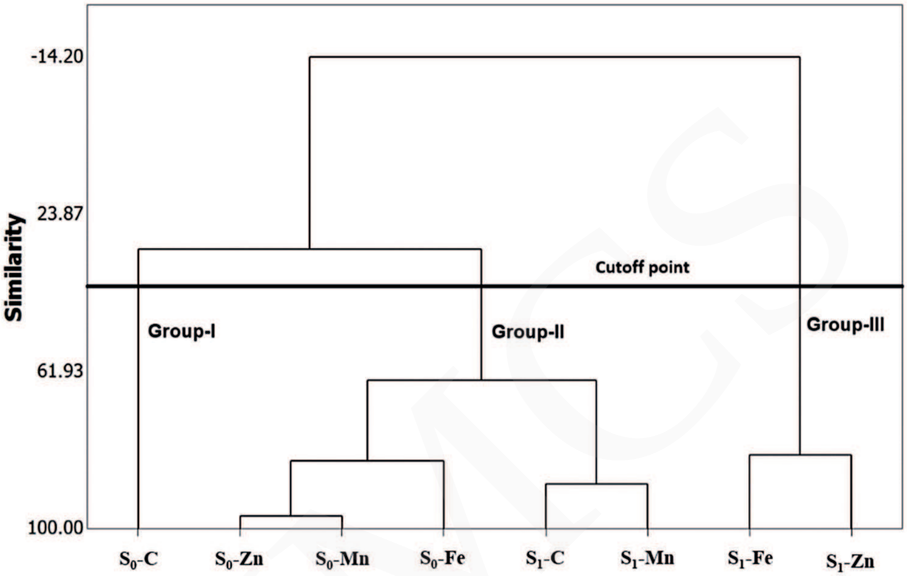

Figure 7. Cluster analysis of nutrient treatments in safflower (*Carthamus tinctorius* L.) grown in highland semi-arid region of Maragheh. C – non-application of nano-metals, S_0 – without sulfur application, S_1 – application of 40 kg ha^{-1} elemental sulfur.

even without the use of sulfur can be somewhat effective. Group III contained the S_1-Zn (application of 40 kg ha^{-1} sulfur along with nano-Zn) and S_1-Fe (application of 40 kg ha^{-1} sulfur along with nano-Fe). The mentioned nutrient treatment resulted in best growth and highest economic yield.

Furthermore, the principle component analysis (PCA) described a suitable amount of the total variation; the correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. In Fig. 8, the most prominent relations are: a strong positive association among oil content and harvest index; also among seed yield, thousand-seed weight, ground cover, plant height, days to maturity, number of capitula, capitulum diameter, number of seeds per capitula and canopy spread as indicated by the small obtuse angles between their vectors (r =cos 0=+1). There was no correlation between the number of days to capitula emergence and oil content or harvest index as indicated by the near perpendicular vectors (r =cos90=0). A strong negative correlation was observed among percentage of unfilled seeds or the number of days to flowering and seed yield as indicated by the wide angle (r =cos180=-1). These results revealed that plans with delayed flowering may show the lower seed yield.

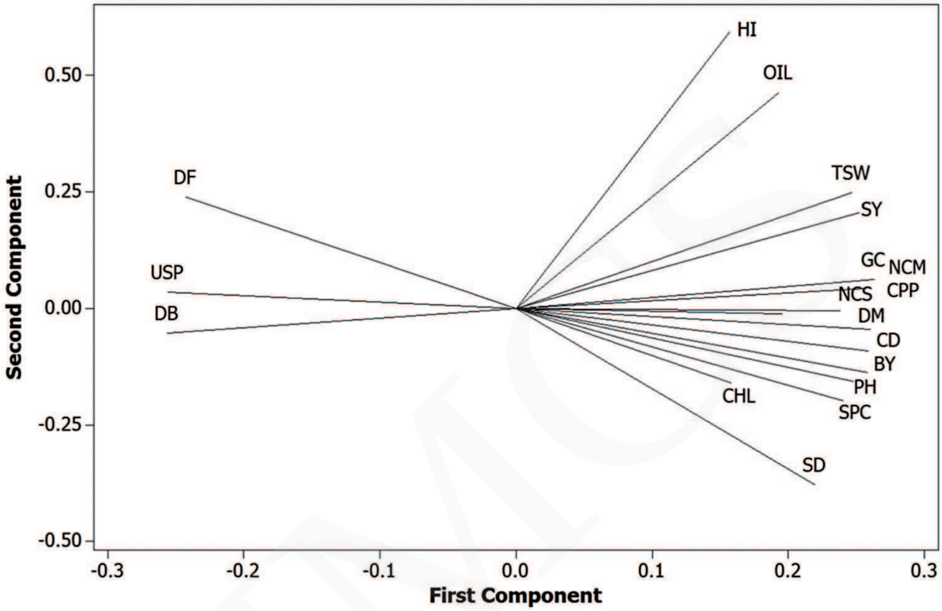

Figure 8. The principle component analysis (PCA) for morphological and agronomical traits of safflower (*Carthamus tinctorius* L.) under different nutrients managements. GC – ground cover, PH – plant height at maturity, SD – stem diameter, chlorophyll content, DCE – number of days to capitulum emergence, DF – days to initiation of flowering, DM – days to maturity, BY – biological yield, CD – capitulum diameter, NCM – number of capitula in main branch, NCS – number of capitula in secondary branches, CPP – number of capitulum per plant, SPC – seed number per capitulum, TSW – thousand seeds weight, USP – unfilled seed percentage, SY – seed yield, HI – harvest index, OIL – oil content.

DISCUSSION

Our finding revealed that application of sulfur considerably increased vegetative growth parameter such as plant height, ground cover, stem diameter and biological yield. The present findings seem to be consistent with other research which found that application of sulfur fertilizers enhanced vegetative parameter in safflower (15). On the other hand, application of nano-metals significantly improved plants growth and this status was more prominent under sulfur applied condition. It was also observed that the highest vegetative growth was obtained by integrated application of sulfur and nano-Zn. It has been revealed that sulfur plays a critical role in chlorophyll formation and can enhance photosynthesis capacity, also it is involved in the activation of many enzymes and intercellular process. Thus it can directly improve the plant growth. Moreover, sulfur is involved in stimulating the synthesis of glutathione and antioxidative processes (4, 20). Moreover, the improvement of plant growth through the sulfur application can be due to its role in modifying the phytohormone biosynthesis. Some of sulfur compounds, such as glutathione and S-adenosyl methionine are precursors of ethylene biosynthesis. Therefore, it seems that there is a crosstalk between sulfur and ethylene signaling and it may have a critical role in regulating plant processes and genes expression (12). The evident impact of sulfur on investigated traits can be due to very low plant-available sulfate concentrations in soil. Although it is reported that sulfur is involved in oil synthesis in the oilseed crops, its effect on total oil content was insignificant.

Mineral nutrition, although contributing a much smaller proportion in terms of weight, is also essential for plant growth. Results of current study showed that nutrient managements can affect sink-source relationship. Generally, the organ in crop plants that synthesizes photoassimilates is named a source, such as the mature leaf. The organ where synthesized assimilates accumulate is known as a sink (18). Application of sulfur and nano-metals increased source capacity through stimulation of the foliage growth. At the same time, fertilizers application improved the sink strength and increased the yield components (number of capitula, seed number per capitulum, seed weight). These results are consistent with finding of Warraich et al. (34) who reported that applied nitrogen fertilizer could improve wheat yield by improving both source and sink efficiency by increasing the leaf area index, relative growth rate, net assimilation rate, grain filling rate and grain filling duration. It has been revealed that nutritional status of sulfur had the strongest effect on the sink strength (9). Our results showed that plants obviously decrease the number of the seed per capitulum and increase the proportion of unfilled seed under conditions of sulfur deficiency.

From the viewpoint of plant production the area-related seed yield was improved equally by both fertilizers and the best performance was recorded for plants grown by integrated application of sulfur and nano-Zn. These results indicate that sulfur can be applied as a common amendment to acidify alkaline soils. Elemental sulfur is oxidized by microbes to produce sulfate (SO_4^{2-}) and H^+, causing a lower pH (21).

Nano-chelated metals are synthetic organic compounds that contain nanoparticles of essential metals in a complex form and protect it from reacting in the soil and forming insoluble precipitates. The mentioned properties increase their efficiency in soil of semi-arid regions. Plants can take up the soluble chelate as complete molecules and then metabolize the metal. Among the micronutrients the highest influence was recorded for Zn and Fe. It has been recognized that iron is considerably less soluble than Zn in soils with a pH value of 8; thus, inorganic Fe contributes relatively little to the Fe nutrition of plants in calcareous soils (11).

Our results showed that application of sulfur or micronutrient fertilizer significantly affected the phonological trend, so that plant grown with utilization of high level of sulfur along with Zn compared to other treatments had a longer reproductive growth. This finding supports previous research into this brain area which showed a significant increase of phenological periods in chickpea by a precise nutrient managements (25). When micronutrient demand and supply are synchronized, there should be no serious negative environmental effects within the agricultural ecosystem. Micronutrients generally bind strongly to the soil and thus are not susceptible to be lost in the environment which minimizes risks of environmental pollution. Furthermore, micronutrients improve crop health, which reduces the need for agrochemicals (pesticides, herbicides, fungicides, etc.). Accumulation in soils due to overuse may cause toxicity problems (3). Overall results showed that safflower plants in semi-arid regions express their full potential only when supplied with integrated balanced fertilizers and non-limiting amounts of water. In this area, due to unfavourable physico-chemical properties of soil, crop responses to nano-metal fertilizers have generally been low and unprofitable to the farmer, unless used in combination with sulfur.

CONCLUSIONS

Soils of Mediterranean semi-arid zones often have high levels of calcium and pH that cause severe micronutrient deficiencies. Our results revealed that application of sulfur fertilizer possibly through rectifying the soil pH can be an effective management option in the reclamation of such soils and improving the availability of essential metals for plants. Sulfur application resulted in a significant increase in seed yield and improved the growth characteristics. Present field survey showed that although the application of nano-chelated metals solely could significantly increase both vegetative and reproductive growth, the best performance was recorded for plants grown by integrated utilization of sulfur and nano-chelated metals. However, these findings suggest that both severe sulfur and essential metals deficiencies may restrict the safflower production in the studied area. The best vegetative growth and the highest seed yield was recorded for plant grown by combined application of sulfur and nano-chelated Zn. Taken together, the application of nano-chelated metals along with sulfur fertilizer in the studied area can considerably improve their efficiency. However, long-term researches are required to precisely evaluate the modification of the soil pH, its effects of micronutrients availability and sulfur effects on these changes in semi-arid regions.

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