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Research paper

Improving some physiological and yield parameters of safflower by foliar sprays of Fe and Zn under drought stress

Kazem Ghassemi-Golezani[1]*, Nian Ardalan[1], Yaghoub Raei[1], and Bahareh Dalil[2]

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[1]Department of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[2]Department of Agriculture, Payame Noor University, Tehran, Iran
*Corresponding author; Email: golezani@gmail.com

Abstract
Water deficiency limits nutrient availability and causes physiological disruptions resulting in decreased crop productivity in the field. The spray of Fe and Zn on drought-stressed plants may reduce some of the detrimental impacts of this stress on crop performance. Thus, this research was laid out as a split-plot design based on a randomized complete block design with three replications to assess the effects of exogenous iron (Fe: 1 g/l) and Zinc (Zn: 1g/l) on safflower (*Carthamus tinctorius* L.) under different irrigation intervals (irrigation after 70, 100, 130, and 160 mm evaporation as normal irrigation, and mild, moderate, and severe water deficits, respectively). Water deficiency decreased green ground cover by reducing plant growth. Leaf water content, chlorophyll content index, capitols per plant, grains per plant, and 1000-grain weight also decreased but leaf temperature increased due to water limitation, leading to a significant loss in the grain yield per unit area under moderate and severe stresses. Foliar sprays of Zn and especially Fe considerably improved the grain yield of safflower under different irrigation intervals, via increasing leaf chlorophyll content, grains per capitol, and grains per plant. However, this superiority in the grain yield decreased with increasing water deficit, which shows that foliar sprays of Fe to a larger extent and Zn to a lesser extent can alleviate some detrimental impacts of mild and moderate water limitations on safflower plants.

Keywords: drought; chlorophyll; green cover; iron; water content; zinc

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Introduction

Safflower is a drought-tolerant plant cultivated in the world's arid and semi-arid lands. Drought is the most limiting factor for the growth and development of crops. Depending on the developmental stage, water stress affects the anatomy, morphology, physiology, and biochemistry of plants. Plants under water shortage tend to close the stomata for diminishing transpiration, leading to an increment in leaf temperature and a decrement in photosynthesis (Zhao *et al*. 2020). Water limitation can reduce the chlorophyll content of plants (Mohammadi *et al.* 2016), which plays a key role in trapping sunlight and converting it into chemical energy. So, any loss in chlorophyll content may reduce photosynthesis (Azhar *et al.* 2011) and consequently grain yield.

Water deficit could reduce the relative water content of plants, leading to stomata closure and thereby lowering CO_2 diffusion to chloroplasts (Muller & Whitsitt 1996). Chloroplast is highly sensitive to different stressful environments such as drought and plays a premier role in modulating stress responses. As drought continues, the stomata closure occurs for longer periods during the day. This can reduce water loss and carbon assimilation rate (Pan *et al*. 2011).

Another most important effect of water deficit is that mobility of some micronutrients will reduce in the soil solution and the plant is more encountered with deficiency of these elements given the root growth restrictions (Amtmann and Blatt 2009). Micronutrients are crucial substances for crop growth. They play a critical role in enhancing leaf area index, thereby increasing light absorption, dry matter accumulation, and economic yield (Ravi 2008). Micronutrients, particularly Fe and Zn, act either as components of different enzymes or as functional, structural, or regulatory cofactors. Thus, they are related to protein synthesis, saccharide metabolism, and photosynthesis (Marschner 1995).

Foliar spray of fertilizers is an efficient way of meeting plants' needs for one or more micro or macronutrients. Microelements such as Zn, Mn, Fe, and Cu could be sprayed on plants to compensate for nutrient deficiency, especially under arid and semi-arid conditions (Saedh *et al.* 2009). It was shown that a small amount of these nutrients as applied by foliar spraying significantly increases the yield and yield components of crops (Saedh *et al*. 2009). Foliar applications of micronutrients have been reported to increase yield in soybean (Zocchi *et al*. 2007) and cotton (Sankarnarayanan *et al*. 2010). It has been observed that Zn increases stress proteins for counteracting drought effects on soybean (Zolfaghari *et al*. 2019). In a study on *Cuminum cyminum*, it was found that applications of Fe and Zn improve plant performance under water deficit (Amirinejad *et al*. 2016). However, the effects of important micronutrients on the physiological performance of safflower under water stress are not clear. Thus, the main objective of this research was to investigate some physiological traits and grain yield of safflower under different irrigation intervals in response to the foliar spray of Fe and Zn.

Materials and Methods

Location and experimental design

This experiment was conducted in April 2016 at the Research Field of the University of Tabriz, Iran (latitude 38.05°N, longitude 46.17°E, altitude 1360 m above sea level). The physical and chemical characteristics of the soil in this area are presented in Table 1. The experiment was undertaken as a split-plot design based on the randomized complete block design with three replicates. Irrigations after 70 (I_1), 100 (I_2), 130 (I_3), and 160 (I_4) mm evaporation from class A pan (as normal irrigation, and mild, moderate, and severe water deficit, respectively) were arranged in the main plots and foliar application of Fe and Zn (using 1 g/l of each of iron chelate and zinc chelate) and untreated plants were allocated in sub-plots. Each plot had 6 rows with 4 m in length and 25 cm distance from each other.

Seeds of safflower (cv. Sina) were sown at 3 cm depth of the soil at a 5 cm distance in rows. All plots were irrigated regularly up to the seedling establishment. Subsequent irrigation intervals were applied according to the irrigation levels to achieve 100% field capacity. Plants were sprayed with water (control), Fe, and Zn at the vegetative (15 leaves) and flowering stages. Weeds were controlled by hand as required. All

Table 1. Soil's physical and chemical properties in the experimental area

Sand (%)	Silt (%)	Clay (%)	pH	EC (dS/m)	P (mg/kg)	K (mg/kg)	Zn (mg/kg)	Fe (mg/kg)
62	22	16	8.2	2.08	8	390	0.92	2.6

physiological traits including green cover, leaf water content (LWC), leaf temperature, and chlorophyll content, were measured at the reproductive stage before irrigation.

Ground green cover

The green cover percentage was measured by viewing the canopy through a wooden frame (50 cm × 50 cm), divided into 100 equal sections. The sections with at least 50% crop green area were counted and recorded.

LWC

Five young fully expanded leaves of a random plant from each plot were cut and immediately placed in a plastic bag within an ice tank and transferred to the laboratory. The fresh weight of leaves was recorded (Fw), and after drying the leaves in an oven at 75 ˚C (Dw), LWC was determined as:

$$LWC (\%) = ((F_W - D_W) / F_W) \times 100$$

Leaf temperature

Leaf temperature (˚C) of upper, middle, and lower leaves of a random plant from each plot was measured by an infrared thermometer (TES-1327). This measurement was carried out at 11 AM just before irrigation.

Chlorophyll content index (CCI)

CCI was recorded by a chlorophyll meter (CCM-200, Opti- Science, USA) in the lower, middle, and upper leaves of a plant from each plot, and the mean value was calculated.

Yield components and grain yield

At maturity, plants in 1 m^2 of the middle part of each plot were harvested and capitols per plant were recorded. Then grains in capitols were detached, weighed and grains per plant, 1000-grain weight, and grain yield per unit area for each plot were recorded.

Statistical analysis

All the data were analyzed by the MSTAT-C software. The mean values for each trait were compared based on Duncan's multiple range test at $p \leq 0.05$, and the figures were drawn by Excel software.

Results

The ground green cover, LWC, leaf temperature, chlorophyll content, capitols per plant, grains per plant, 1000-grain weight, and grain yield per unit area were significantly affected by the irrigation intervals. However, foliar sprays of Fe and Zn had only significant effects on leaf chlorophyll content, grains per capitol, grains per plant, 1000-grain weight, and grain yield. The interaction of irrigation × nutrients was only significant for grain yield per unit area (Table 2).

Table 2. Analyses of variance for some physiological traits and grain yield of safflower affected by water supply and foliar spray of Fe and Zn

Source of variance	df	GC	LWC	LT	CCI	CN	GPC	GPP	TGW	GY
Replication	2	802.9	17.4	2.6	72.7	11.7	1.8	2418.7	36.4	3647.7
Irrigation (I)	3	1320.7*	575.0**	809.4**	366.3**	235.1**	46.1	25891.7**	92.1**	68708.4**
Error a	6	147.8	14. 0	14.6	3.5	7.03	11.8	1595.8	5.1	2928.4
Nutrient (N)	2	25.9	33.3	1.4	164.1**	0.7	66.1*	4906.7*	85.4**	17947.0**
I × N	6	95.9	26.5	2.3	2.3	4.9	11.4	25465.3	6.2	4569.8*
Error b	16	78.6	15.7	2.7	13.7	2.9	10.6	1147.3	4.1	1564.9
CV (%)		16.7	6.0	5.5	10.1	16.3	24.9	25.7	5.1	21.8

* and **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; GC: ground cover, LWC: leaf water content, LT: leaf temperature, CCI: chlorophyll content index, CN: capitol number, GPC: grains per capitol, GPP: grains per plant, TGW: 1000-grain weight, GY: grain yield

Ground green cover percentage linearly decreased with decreasing water supply. However, this reduction was not significant under mild (I_2) and moderate (I_3) water deficits. The differences in percentage ground green cover between mild (I_2) and moderate (I_3) and also between moderate (I_3) and severe (I_4) stresses were not statistically significant. Nevertheless, ground cover under I_2, I_3, and I_4 was reduced by 18.2%, 32.9%, and 39.7%, respectively, although this reduction in comparison with normal irrigation (I_1) was only significant under severe stress (I_4) (Figure 1).

LWC was considerably reduced as a consequence of decreasing water availability, although differences between two consecutive levels of irrigation were not statistically significant (Figure 2A). Leaf temperature was linearly increased with increasing irrigation intervals, but no significant differences between I_1 and I_2 and also between I_2 and I_3 were found. Leaf temperature under well-watering (I_1) was almost 20 °C lower than that under severe water stress (I_1) (Figure 2B).

The mean CCI was significantly decreased by increasing the irrigation intervals, although there was no significant difference between normal irrigation (I_1) and mild water stress (I_2) (Figure 3A). Foliar spray of Fe resulted in the highest leaf chlorophyll content, followed by Zn spray, with no significant difference between these two treatments (Figure 3B).

Capitols per plant (Figure 4A), grains per plant, 1000-grain weight (Figure 5), and grain yield (Figure 6) of safflower were generally reduced by increasing water limitation. Capitols per plant under normal irrigation (I_1) and mild stress (I_2) were higher than that under moderate (I_3) and severe stresses (I_4), with no significant difference between I_1 and I_2 and also between I_3 and I_4 (Figure 4A). The highest grains per capitol was obtained from plants sprayed with Fe, followed by Zn. There was no significant difference between the latter treatment and water spray (Figure 4B).

The highest and the lowest number of grains per plant were achieved under I_1 and I_4, respectively. In general, changes in capitols per

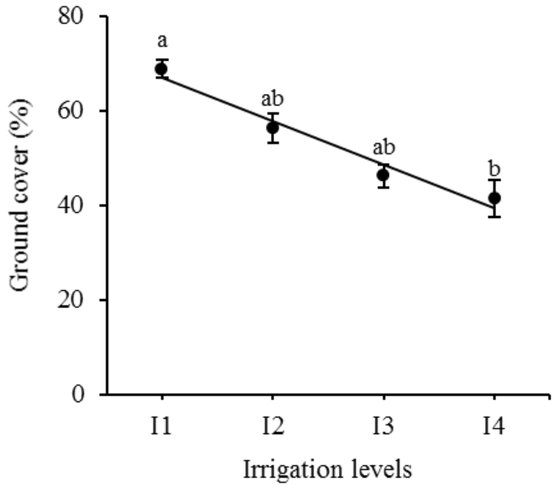

Figure 1. Changes in the ground green cover of safflower in response to water stress; I_1, I_2, I_3, I_4: irrigation after 70, 100, 130, and 160 mm evaporation, respectively; different letters indicate a significant difference at $p \leq 0.05$.

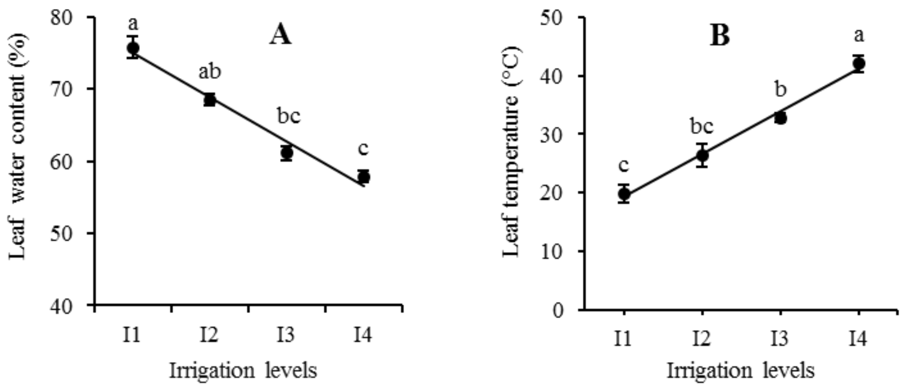

Figure 2. Changes in leaf water content (A) and temperature (B) of safflower under different irrigation intervals; I_1, I_2, I_3, I_4: irrigation after 70, 100, 130, and 160 mm evaporation, respectively; different letters indicate a significant difference at $p \leq 0.05$.

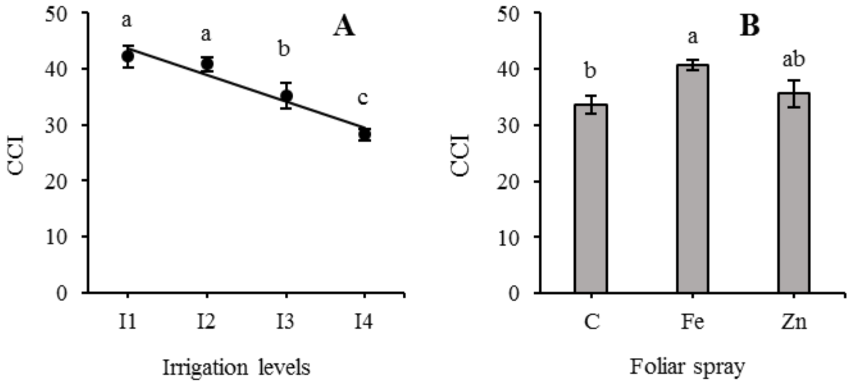

Figure 3. Changes in chlorophyll content index (CCI) of safflower in response to water stress (A) and foliar spray of micronutrients (B); I_1, I_2, I_3, I_4: irrigation after 70, 100, 130, and 160 mm evaporation, respectively; different letters indicate a significant difference at $p \leq 0.05$.

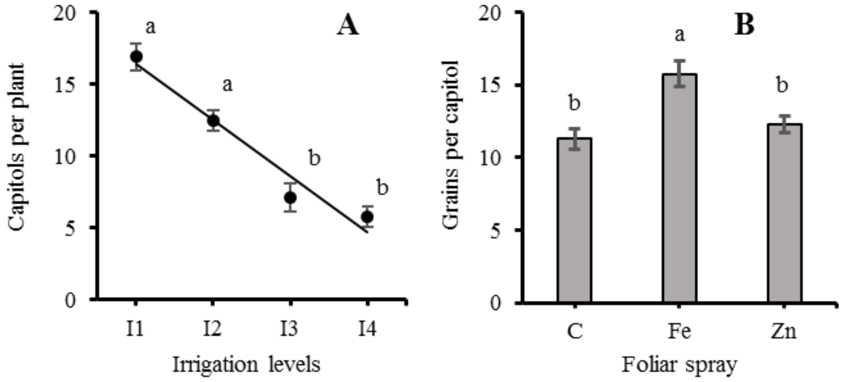

Figure 4. Capitols per plant of safflower under different irrigation intervals (A), and grains per capitol affected by foliar spray of Fe and Zn (B); different letters indicate a significant difference at $p \leq 0.05$.

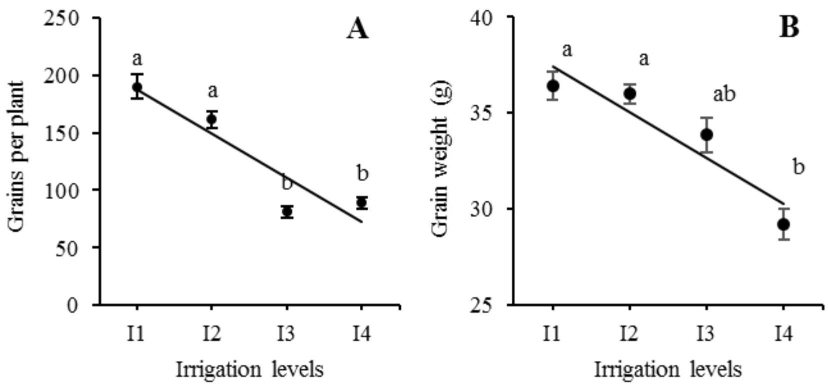

Figure 5. Grains per plant (A) and 1000 grain weight (B) of safflower under different irrigation intervals; different letters indicate a significant difference at $p \leq 0.05$.

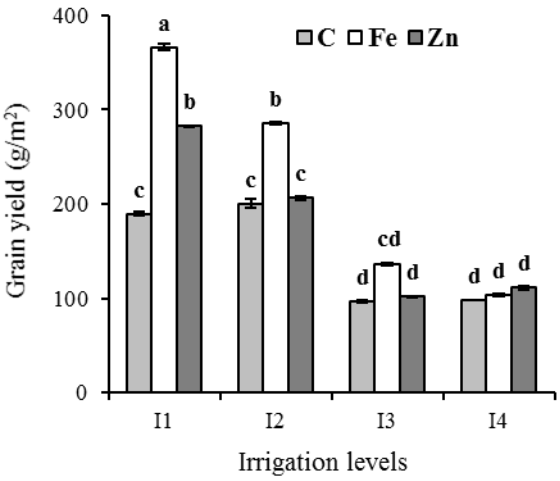

Figure 6. Mean grain yield of safflower under different irrigation intervals affected by foliar spray of Fe and Zn; I_1, I_2, I_3, I_4: irrigation after 70, 100, 130, and 160 mm evaporation, respectively; different letters indicate a significant difference at $p \leq 0.05$.

plant (Figure 4A) and grains per plant (Figure 5A) under water stress were similarly declined with enhancing irrigation intervals. Mean 1000-grain weight under severe water stress (I_4) was statistically lower than the other irrigation levels. However, there were no significant differences in grain weight of plants under I_1, I_2, and I_3, and also under I_3 and I_4 (Figure 5B).

Similar to grains per capitol, the highest number of grains per plant was recorded for the plants sprayed with Fe, followed by Zn, with no significant difference between the latter treatment and water spray. Plants sprayed with Fe showed 34.3% superiority in grains per plant, compared with untreated plants. However, there was no significant difference between plants sprayed with Fe and Zn and also between Zn and water sprays (Figure 7A). Although the exogenous application of Fe comparatively enhanced the grain weight of safflower plants, this advantage was only significant in comparison with water-sprayed plants (Figure 7B).

Increasing water limitation led to a 17.35%, 63.31%, and 62.79% reduction in grain yield, compared with well watering (I_1). The highest grain yield per unit area under I_1, I_2, and I_3 were recorded for the Fe treatment. This superiority decreased with decreasing water availability. Foliar spray of Zn also significantly enhanced grain yield under normal irrigation, but not under limited irrigations. Grain yield under severe stress was statistically similar for control and Fe and Zn treated plants (Figure 6).

Discussion

The reduction in ground green cover under water stress (Figure 1) could be attributed to the competition of plants for water and nutrients under stress (Xu *et al.* 2010). Similar results were reported for chickpea (Ghassemi-Golezani *et al*. 2012), dill (Ghassemi-Golezani and Solhi-Khajemarjan 2021), and maize (Saseendran *et al*. 2015). Environmental stresses such as water limitation can decrease ground green cover by reducing the number of green leaves, leaf area, and leaf area index, as water is essential for the metabolism and translocation of nutrients in the plants (Sales *et al*. 2013). The decline in ground green cover can ultimately reduce photosynthesis and grain yield of crops (Ghassemi-Golezani *et al*. 2012).

Variations in LWC under water limitation (Figure 2A) may have resulted from the differences in cell wall integrity and stability under water stress (ElBasyoni *et al*. 2017). Declining LWC due to water stress could be related to an imbalance between water loss from the leaves due to transpiration and water uptake by the roots (Jones 2007). Safflower is considered to be a drought-tolerant plant and there is a direct relationship between LWC and drought resistance (Mohammadi *et al*. 2016). It has been stated that high LWC is a mechanism of drought resistance rather than drought escape and it is believed that high LWC is the result of higher osmotic regulation of tissue with lower elasticity (Ritchie *et al.* 1990). Augmentation of leaf temperature under water stress (Figure 2B) was the result of the decline in LWC (Figure 2A) and stomata closure. In general, water limitation decreases

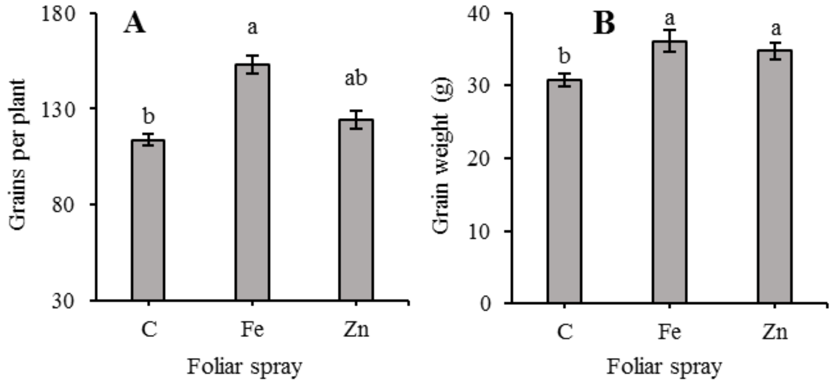

Figure 7. Grains per plant (A) and 1000 grain weight (B) of safflower affected by foliar spray of Fe and Zn; different letters indicate a significant difference at $p \leq 0.05$.

stomatal conductance due to the closure of stomata, which helps to reduce water loss due to transpiration. This reduction in transpiration enhances leaf temperature (Zhao *et al.* 2020). Increasing leaf temperature due to water limitation has also been reported in maize (Dalil and Ghassemi-Golezani 2012) and lentils (Chadordooz-Jeddi *et al*. 2015). Drought and heat stress frequently combine to scorch leaves. Heat stress damages the membrane, especially the thylakoid membrane, thereby diminishing the activities of membrane-associated electron carriers and enzymes (Rexroth 2011). In addition, rising leaf temperature due to dehydration can reduce the activation state of Rubisco in both C_3 and C_4 plants. The reduction in active Rubisco could be accounted for by the temperature response of net photosynthesis (Salvucci and Crafts-Brandner 2004). It was shown that high leaf temperature caused by water limitation had negative effects on the photochemical efficiency of PSII (Ghassemi-Golezani *et al*. 2018). Leaf temperature was negatively correlated with Fv/Fm and positively with non-photochemical quenching (Shahenshah and Isoda 2015). Photosynthesis, as one of the most heat- sensitive processes, can be completely inhibited by high temperature before other symptoms of the stress are detected (Camejo *et al*. 2005).

Reduction in CCI (Figure 3A) due to water deficit may partly result from low water content and high leaf temperature (Figure 2). Studies on chlorophyllase and peroxidase revealed that the decrease may be attributed to the accelerated breakdown of chlorophyll rather than its slow synthesis (Kaewsuksaeng 2011). It was suggested that a high leaf temperature could affect the destruction of chlorophyll through a series of physiological and photochemical reactions, i.e., limitation of carbon dioxide fixation resulting from inactivation of photosynthetic enzymes, then increment of active oxygen, induced by excess excitation energy (Smirnoff 1993). Reductions in leaf CCI (Figure 3A) can strongly limit light interception, photosynthesis, and consequently, grain yield (Ghassemi-Golezani *et al*. 2010). Increasing chlorophyll content by Fe spray might be due to the participation of iron in the formation of chlorophyll. Iron has a role in the porphyrin structure of chlorophyll and thus it is a principal component of the chloroplasts. Therefore, Fe has many essential roles in plant growth and development including chloroplast development, chlorophyll synthesis, and thylakoid synthesis (Rout and Saho 2015). Zinc also had a positive effect on CCI and it might be because of its essential roles in the plant growth and development including stomatal conductance, the maximum quantum yield of photosystem II (Wang *et al*. 2009), biomass production, chlorophyll synthesis, pollen function, fertilization, RNA metabolism, and protein and DNA formation (Cakmak 2008). Therefore, iron and zinc have important roles in chlorophyll production, and any lack of these microelements can diminish chlorophyll formation.

Reducing grains per plant and 1000-grain weight due to water deficit (Figure 5) was directly associated with diminishing green ground cover (Figure 1), LWC (Figure 2A), CCI (Figure 3A), and also with enhancing leaf temperature (Figure 2B) under drought stress. Reduction in grains per plant (Figure 5A) is the result of decreasing capitols per plant caused by water stress (Figure 4A). This was supported by the previous report on safflower (Ghassemi-Golezani *et al*. 2016). Drought stress diminishes dry matter production (Ghassemi-Golezani *et al*. 2016), grain filling duration (Ghassemi-Golezani *et al*. 2015), the photosynthate mobilization (Kumar 2016), and eventually the grain weight. The impact of water stress at reproductive stage on grain weight can be explained by a reduction in assimilate production during grain filling period (Ghannoum 2009).

The higher grain number per plant in the Fe-sprayed plants (Figure 6A) could have resulted from higher grains per capitol (Figure 4B). Iron may result in higher dry mass (Choudhary *et al*. 2017), because of improvement in CCI. Tripathi *et al*. (2017) also reported that iron plays an irreplaceable role in alleviating harmful impacts of drought stress on plants. Zinc application also slightly enhanced grains per plant (Figure 6A), due to the contribution to pollination through its impact on pollen tube formation (Pandey *et al*. 2006). In addition, foliar spray of Zn can play an important role in decreasing drought effects by interacting with phytohormones, increasing the expression of stress proteins, and stimulating the activities of antioxidant enzymes (Hassan *et al*. 2020). According to Amirinejad *et al*. (2016), exogenous Fe and Zn improve biochemical parameters in cumin under water stress.

The high chlorophyll content of the plants treated with Fe and Zn (Figure 3B) can be the main reason for enhancing the grain weight of safflower (Figure 6B). Foliar sprays of Fe can affect catalase and cytochrome oxidase enzymes and chlorophyll formation (Kumar 2016). The positive effects of micronutrients on chlorophyll content (Figure 3B) suggest that micronutrient application can improve the grain weight through increasing assimilate supply (Janmohammadi *et al*. 2016). Increasing grain yield per unit area due to foliar sprays of Fe and Zn under different irrigation intervals (Figure 7) is the result of improving grains per plant and 1000-grain weight (Figure 6). Increasing CCI by foliar sprays of Fe and Zn (Figure 3B) positively influences the photosynthesis machinery of the plants, which can enhance assimilate production and partitioning, and consequently grain yield per unit area.

Loss of 17-63% in grain yield due to water deficit (Figure 7) was associated with the reduced grains per plant and the grain weight of safflower under stress (Figure 5). Water stress causes a reduction in leaf area index, carbon allocation, photosynthesis, and consequently grain yield (Yordanov *et al*. 2000). Absorption of incident PAR may be reduced by temporary leaf wilting and early leaf senescence which potentially reduces the grain yield of crops (Ghassemi-Golezani *et al*. 2012).

Conclusion

Water deficit caused a reduction in ground green cover, LWC, and CCI, leading to a considerable loss in capitols per plant, grains per plant, grain weight, and consequently grain yield per unit area. However, foliar sprays of Zn and particularly Fe improved most of these traits, except capitols per plant. The superiority of these treatments in grain yield per unit area was decreased with decreasing water availability. Nevertheless, foliar sprays of Fe and Zn mitigated some of the deleterious effects of water limitation on the field performance of safflower. Future works with different concentrations of exogenous nutrients on this and other plant species under various levels of water deficits may reveal more beneficial effects of these treatments on crop growth and productivity under stressful conditions.

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Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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بهبود برخی پارامترهای فیزیولوژیکی و محصول گلرنگ با محلول‌پاشی آهن و روی تحت تنش خشکی

کاظم قاسمی گلعذانی[۱*]، نیان اردلان[۱]، یعقوب راعی[۱] و بهاره دلیل[۲]

۱- گروه اکوفیزیولوژی گیاهی، دانشکده کشاورزی، دانشگاه تبریز، تبریز

۲- گروه کشاورزی، دانشگاه پیام نور، تهران

*مسئول مکاتبه؛ Email: golezani@gmail.com

چکیده

کمبود آب دسترسی به عناصر غذایی را محدود می‌کند و باعث اختلالات فیزیولوژیکی می‌شود. در نتیجه قابلیت تولید محصول زراعی در مزرعه کاهش می‌یابد. پاشیدن آهن و روی بر گیاهان تحت تنش خشکی شاید برخی از اثرات مضر این تنش روی عملکرد گیاه زراعی را کاهش دهد. بنابراین، این پژوهش به صورت طرح اسپلیت پلات بر پایه طرح بلوک‌های کامل تصادفی با سه تکرار برای ارزیابی اثرات آهن (1 g/l) و روی (1 g/l) خارجی روی گلرنگ (*Carthamus tinctorius* L.) در فواصل مختلف آبیاری (آبیاری پس از ۷۰، ۱۰۰، ۱۳۰ و ۱۶۰ میلی‌متر تبخیر به ترتیب به عنوان آبیاری معمول، و کم‌آبی‌های ملایم، متوسط و شدید) طراحی شد. کمبود آب با کاهش رشد گیاه درصد پوشش سبز را کاهش داد. به دلیل محدودیت آب، محتوی آب برگ، شاخص محتوای کلروفیل، تعداد کاپیتول در بوته، تعداد دانه در بوته و وزن هزار دانه نیز کاهش یافتند، ولی دمای برگ افزایش یافت که منجر به افت معنی‌دار محصول دانه در واحد سطح تحت تنش‌های متوسط و شدید شد. محلول پاشی روی و به ویژه آهن با افزایش محتوای کلروفیل برگ، تعداد دانه در کاپیتول و تعداد دانه در بوته موجب بهبود قابل ملاحظه محصول دانه گلرنگ در فواصل مختلف آبیاری شد. اما، این برتری در محصول دانه با افزایش کمبود آب کاهش یافت که نشان می‌دهد محلول پاشی آهن به مقدار بیشتر و روی به مقدار کمتر می‌توانند برخی از اثرات زیان‌بار محدودیت‌های آبی ملایم و متوسط روی گیاه گلرنگ را کاهش دهند.

واژه‌های کلیدی: آهن؛ پوشش سبز؛ خشکی؛ روی؛ کلروفیل؛ محتوای آب