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Magnesium Nanoparticles Improve Grain Yield, Oil Percentage, Physiological, and Biochemical Traits of Sunflower (*Helianthus annuus* L.) under Drought Stress

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is an oil grain crop of subtropical and tropical areas with arid to semi-arid climate and its production is affected by drought at the grain filling and flowering stages. Given the key role of Magnesium (Mg) in many key metabolic processes, it seems that Mg Nanoparticles (NPs) can improve the sunflower performance in drought stress. This study was carried out as a split-split plot in a randomized complete block design with three factors, including irrigation regimes (drought and normal), cultivars (Barzagar, Farokh, Ghasem, and Shams), and Mg NPs application (0.25 g L^{-1}), and time (flowering and grain filling stages), in three replications in the field during 2017-19 seasons. Several physio-biochemical traits along with grain yield and oil percentage were measured. Based on the results, Mg NPs spraying increased the relative water content, chlorophyll and carotenoid, soluble carbohydrates, and antioxidant enzymes activity under drought. Moreover, Mg NPs spraying decreased electrolyte leakage and malondialdehyde content in the stressed plants and slightly increased grain yield and oil percentage. Overall, our findings suggest that Mg NPs can improve sunflower performance under drought by several mechanisms including improved antioxidant system, enhanced photosynthetic pigments, and increased primary metabolites.

Keywords: Abiotic stress; Antioxidant enzymes, Oil grain crop, Sunflower cultivars.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oil grain crop with 45 million metric tons production, globally cultivated on 25 million hectares. This oil grain possesses 8.5% share in the world market of oil grain and contains 18–20% protein and 40–50% oil, thus it is important for narrowing the gap between edible oils production and consumption in various countries (Hussain *et al.,* 2018). This plant belongs to subtropical and tropical areas and is grown mostly in arid to semi-arid climates; therefore, it is affected by unfavorable environmental factors like drought and heat (Robert *et al.,* 2016; Pekcan *et al.,* 2015). From early flowering to achene filling, sunflower is highly susceptible to heat and drought stresses, due to incompetence in controlling the leaves development and transpiration rate under limited moisture in soil, although this oil grain is somewhat drought tolerant due to its ability to escape from drought (Aboudrare *et al.,* 2006). A decrement in the moisture of soil results in the leaf wilting, which leads to considerable yield loss in semi-arid regions with inadequate rainfall (García-López *et al.,* 2014). So far, many researches have revealed that drought reduces oil yield and quality in sunflower (Oraki and Aghaalikhana, 2012). The effect of drought on productivity, however, is not the same for all the growth stages of sunflower. Drought at grain filling and flowering is the key

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factor causing up to 50% loss in sunflower yield (Hussain *et al.*, 2008). Drought stress in early season inhibits leaf and stem extension, whereas at flowering it leads to empty achene production due to the failure in pollen fertility (Fulda *et al.*, 2011; Aboudrare *et al.*, 2006). More accessible moisture at earlier stages of growth leads to favorable vegetative growth, while subsequent drought at seed filling decreases the sunflower yield due to high demand for transpiration (Aboudrare *et al.*, 2006).

To date, several researches have revealed that drought tolerance in crops could be improved through Nanoparticles (NPs) spraying. For instance, the positive effects of Si and Fe NPs on several physiological traits under drought have been recorded by Gunes *et al*. (2008) and Davar *et al*. (2014). Ag NPs were also utilized to reduce the adverse effects of drought (Hojjat, 2016). ZnO NPs have been shown to improve drought tolerance by enhancing CAT and SOD activities (Taran *et al.*, 2017). Thus, NPs spraying have a potential to increase relative water content of leaves, stabilize the photosynthetic pigments content, reduce lipid peroxidation, increase antioxidative enzymes activity, and eventually improve drought tolerance. However, there is no report on evaluation of the effect of Mg NPs on plant performance under drought. Magnesium plays several important roles in plants and crops. Specific metabolic reactions and processes that are affected by Mg include: (1) Photo-oxidation in leaves tissue, (2) Production of reactive oxygen species, (3) Utilization and partitioning of photo-assimilates, (4) Phloem loading, (5) Chlorophyll generation, (6) Protein synthesis, (7) Photosynthetic CO_2 fixation, and (8) Photo-phosphorylation (Cakmak and Yazici, 2010). Thus, many key biochemical and physiological processes in crops and plants are influenced unfavorably by Mg shortage, resulting in loss in growth and performance. Also, it is documented that drought stress has a negative effect on the Mg uptake and sunflower response (Canavar *et al.*, 2014).

Given the key role of Mg as mentioned above, it seems that Mg NPs foliar application can improve the drought-tolerance in sunflower. Therefore, we aimed to investigate, for the first time, the idea of spraying Mg NPs to enhance the performance of sunflower under drought stress based on drought tolerance-related biochemical and physiological traits along with plant performance.

MATERIALS AND METHODS

Plant Material

To assay the effect of spraying of Mg NPs on the performance of sunflower under drought stress, a total of four cultivars of sunflower, including Barzagar, Farokh, Ghasem, and Shams were obtained from the Seed and Plant Improvement Institute (SPII), Iran.

Climatology Data

Mean temperature and monthly total rainfall for each month during 2017-2018 and 2018-2019 are according to the data at the meteorological station in the Islamic Azad University, Tehran, Iran (latitude at 32 degrees and 16 minutes and longitude of 48 degrees and 25 minutes, and 82 meters above sea level) (Figure 1).

Drought Stress

The irrigation regimes were as follows: (1) Normal- No drought stress and soil moisture was at field capacity (100%) in all growth periods, and (2) Drought stress- Soil moisture was kept at 60% of field capacity. The drought stress was applied to the sunflower cultivars at the five-leaf stage. To achieve this goal, the stress test plots were irrigated every two weeks until the field capacity reached 60% and maintained at this position (Xu *et al*., 2019). Soil FC was

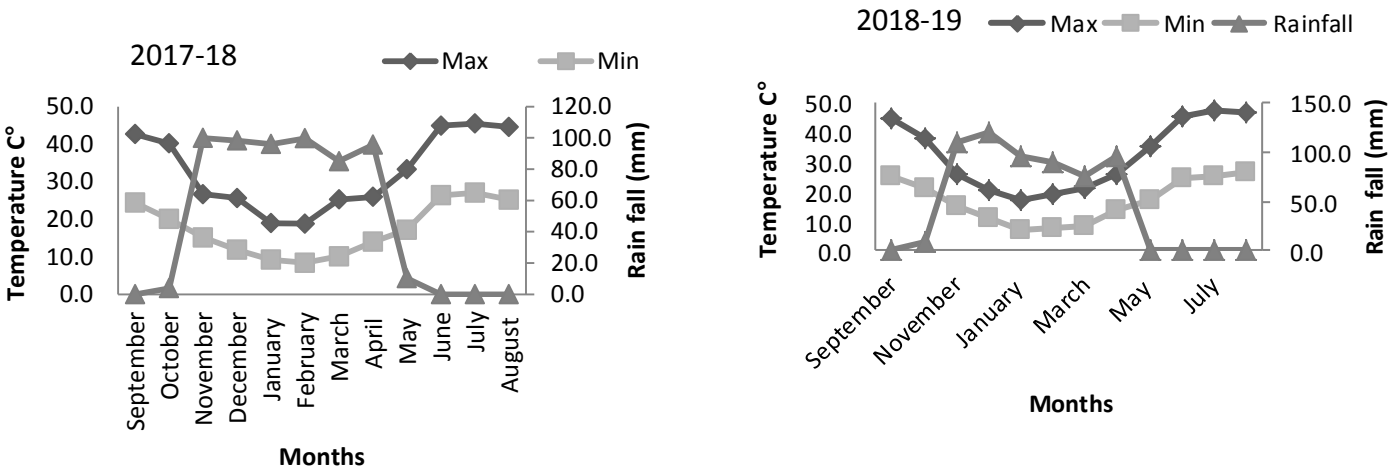


Figure 1. Monthly total rainfall, Minimum (Min) and Maximum (Max) averages of temperature in 2017-2018 and 2018-2019.

determined by using the pressure plate method (Fulda *et al.*, 2011).

Nanoparticles

Magnesium nanoparticles (Mg NPs) were purchased from the NANOSANY Corporation, Iran. Table 1 illustrates the features of Mg NPs based on the report of this company. Mg NPs were suspended in deionized water and then sonicated (40kHz, 100W) for 30 minutes. To avoid aggregation of the Mg NPs, the suspension was mixed continuously by a magnetic stirrer before application (Davar *et al.*, 2014). Mg NPs (0.25 g L^{-1}) foliar application was applied in the grain filling and flowering stages.

Experimental Design

This study was carried out as a split-split plot in a randomized complete block design with three factors and three replications in the oil grain field of Seed and Plant Improvement Institute (SPII) during 2017-2019. The three factors in this experiment included: Factor I (A) as drought stress at two levels including Normal (A1), as irrigation time of experimental plots based on 85% FC since planting until the end of growing season, and Drought (A2), with the irrigation time of experimental plots based on 60% FC; Factor II (B) as sunflower cultivars including Barzegar (B1), Farrokh (B2), Qassim (B3), and Shams (B4); Factor III (C) as application time of Mg NPs (0.25

Table 1. Characteristics of Mg NPs used in the current study.

Particle	Size (nm)	Purity (%)	Surface area (m^2 g^{-1})	Shape	Bulk density (g cm^{-3})	True density (g cm^{-3})
Mg	20	98	> 60	Polyhedral	0.145	3.58

g L^{-1}) at three levels, No spraying (C1), Mg NPs spraying at flowering stage (C2), and Mg NPs spraying at the grain filling stage (C3).

The field in this experiment had a latitude of 50° 55' E, 35° 50' N with an altitude of 1,321 meters above sea level. Before starting the experiment, composite sampling was taken from the field soil and different chemical and physical characteristics of the soil were determined (Table 2). Based on the soil analysis, the NPK fertilizer, including 150 kg of urea, 50 kg of phosphate, and 100 kg of potassium sulfate, was applied to the plots before the experiment. In order to eliminate marginal effects, two sidelines and 1.5 m from both sides of the plots were considered as margins and were not used in the sampling (Oraki and Aghaalikhana, 2012). Each block consisted of 24 plots, each plot was 3×4 m in size, and 96 plants were assayed per plot.

Grain Yield and Oil Percentage

Grain yield was calculated as kg/ha. The oil percentage was measured by using Soxhlet extractor apparatus. For this purpose, some grains of each test unit was kept in the oven for one day at 70°C and then powdered with a mill. One gram of the powdered grains was weighed accurately. The samples were placed in Soxhlet for 8 hours by using hexane to completely remove the oil. The samples then were removed from the extractor apparatus and placed in the oven for overnight at 70 °C, and weighed eventually. The oil percentage was measured via the following equation (Pekcan *et al.*, 2015):

%Oil= [(Sample weight difference after and before oil extraction)/(Initial weight)]×100

Physio-Biochemical Measurements

Total protein content of leaf was determined based on Bradford (1976) by bovine serum as standard. In this method, 1 mL of Bradford solution was poured into 1.5 mL tubes and then 50 µL of the extract was added and, after 30 minutes, absorbance was read at 595 nm wavelength.

For antioxidant enzymes activates being estimated, 0.5 g of fresh leaves were homogenized in 8 mL of phosphate buffer (50 mM, pH 7.7) by using pestle and mortar on ice. Homogenate then was centrifuged at 10,000×g at 4°C for 20 minutes, and supernatant was utilized for measuring the activity of Ascorbate Peroxidase (APX) based on Chen *et al.* (2010), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPX) based on Wu *et al.* (2003).

Relative Water Content (RWC), as a measure of the plant water status, was estimated according to Canavar *et al.* (2014). After determination of the fresh weight, flag leaf samples (0.5 g) were saturated in 100 mL distilled water for 24 hours at 4°C under the dark, then, their turgid weight was recorded. In the following, samples were oven-dried at 70°C for 48 hours and their dry weight was recorded. Finally, RWC was calculated as follows:

RWC= [(Fresh weight-Dry weight)/(Turgid weight–Dry weight)]×100.

To measure the Electrolyte Leakage (EL)

Table 2. The soil analysis results of field studied.

Total Nitrogen (%)	Available Phosphorus (mg kg^{-1})	Absorbable Potassium (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	Mn (mg kg^{-1})	Acidity of saturated extract (pH)	Electrical Conductivity of saturated extract (EC) (ds m^{-1})	Organic carbon (%)	Soil texture (Loam)		
									Clay (%)	Silt (%)	Sand (%)
0.127	14.1	180	8.2	4.3	2.3	8.3	0.87	1.53	33	36	31

(Lutts et *al.*, 1996), plant tissue samples (0.3 g) were washed with deionized water, placed in tubes containing 15 mL deionized water and incubated for 2 hours at 25°C, and the Electrical Conductivity of the solution (EC_1) was determined. In the following, samples were autoclaved at 100°C for 30 minutes, and the final Conductivity (EC_2) was recorded after their temperature cooled to 25°C. Given the EC_1 and EC_2 values, the EL was calculated as follows:

$EL= (EC_1/EC_2)\times100$.

Chlorophyll and carotenoid content was also determined by the method described by Arnon (1949). In this method, leaf sample (0.1 g) was thinned in mortar with 3 mL of 80% acetone, and the extract volume was increased to 15 mL. Then, the extract was filtered through centrifugation for 10 minutes at 5,000 rpm, and absorbance was read at 645, 663, 480, and 510 nm through Spectrophotometer (Shimadzu UV-160). The amount of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid were calculated according to the formula of Arnon (1949).

To measure the proline content, based on the method described by Bates *et al.* (1973), 0.2 g leaf was placed in 10 mL sulfosalicylic acid 3% solution, and the resulting mixture was completely homogenized in mortar. Then, 2 mL of the mixture was mixed with 2 mL Nine Hydrine, and then 2 mL acetic acid was added to each tube. The samples were placed in the hot bath 100°C for one hour and immediately placed in the ice bath for a few min. In the following, 4 mL toluene was added to each tube and the samples were mixed for 15 seconds to become uniform completely. Finally, the supernatant was used to determine the proline concentration, according to the proline standard curve in spectrophotometer at 520 nm.

The soluble carbohydrates content was estimated according to Comis *et al.* (2001). The 0.5 g dried leaf was homogenized in 10 mL of 80% ethanol, then centrifuged for 15 minutes at 8,000 rpm. Later, the supernatant was filtered through 0.2 micrometer and injected into the HPLC (Unickam-crystal 200, UK) to separate the sugars. The fixed phase consisted of the spherisorb column with 15 cm length and 4.6 mm diameter, containing 0.3-microns particles. The mobile phase also consisted of sodium citrate buffer (pH= 5.5) and ultra-pure acetonitrile (1:99) with a flow rate of 2 mL per minute. Finally, sugars types and amount in the samples was determined based on retention times using standard samples (Comis *et al.*, 2001).

For estimation of the level of Malondialdehyde (MDA), as output derived from lipid peroxidation, the cell extracts were made through 1.0 g of fresh tissue following the method elucidated by Heath *et al.* (1968). The absorbance at 532 and 600 nm was recorded for the extract. Average of the readings in triplicate was utilized for estimating the MDA level by extinction coefficient of 155 mM^{-1} cm^{-1} and the formula as follows:

$MDA\ (nM)= \Delta A_{(532-600)}/1.56\times10^5$.

Here ΔA indicates the wavelength difference between the 532-600 nm.

Statistical Analysis

Analysis Of Variance (ANOVA) along with Duncan's multiple range test ($P< 0.05$) were carried out through Statistical Analysis System version. 9.4.

RESULTS

Our results showed that drought significantly altered the agronomical, physiological, and biochemical traits in sunflower. Mg NPs foliar application, in turn, significantly improved grain yield in the stressed plants when compared to the control (Figure 2-a), such that the Mg NPs spraying increased the yield up to ~1.2 fold over that of non-treated plants. The oil percentage was significantly raised by Mg NPs supply under drought. The NPs treatment enhanced the oil percentage by 1.1-fold in flowering and grain filling stages as compared to the control (Figure 2b). Foliar application of Mg NPs increased total protein insignificantly (Figure 2-c).

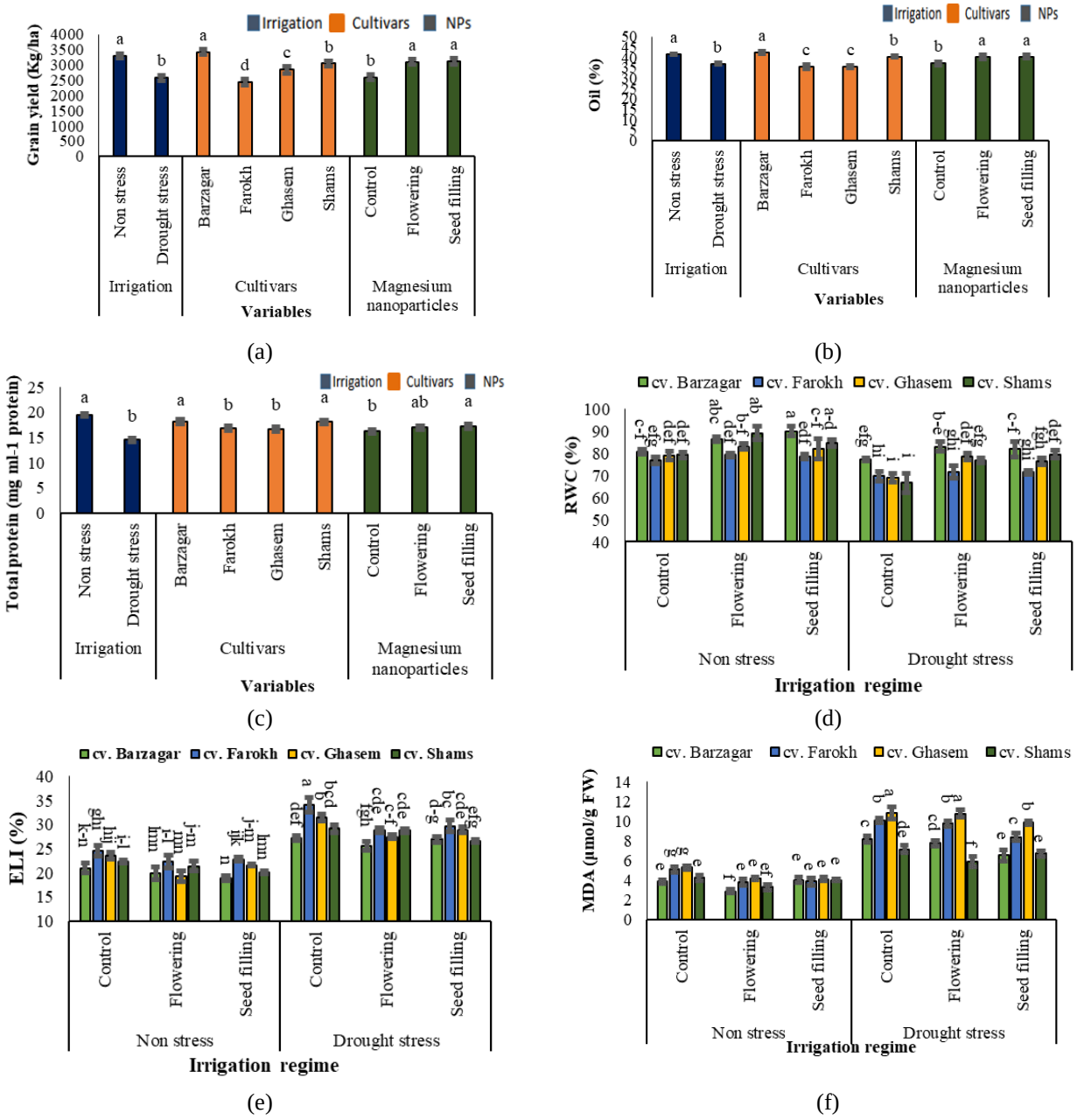
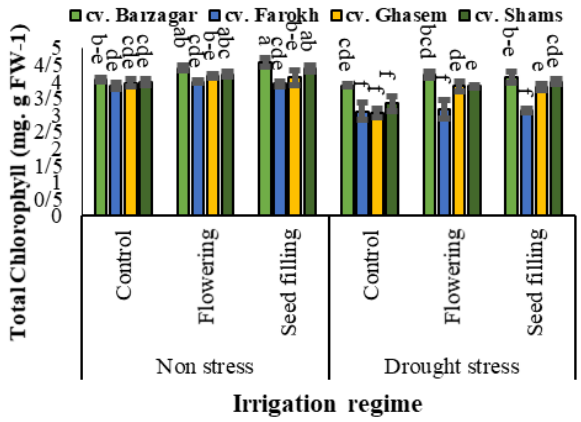


Figure 2. The effect of Nano-magnesium foliar application on seed yield (a), oil percentage (b), total protein (c), RWC (d), ELL (e), MDA (f), total chlorophyll (g), a chlorophyll (h), b chlorophyll (i), carotenoid (j), proline (k), soluble carbohydrates (l), APX (m), GPX (n), and SOD (p) activity in the four sunflower cultivars. Columns with at least one letter in common did not have a statistically significant difference based on the Duncan test at the 0.05 level.

Figure 2 Continued…



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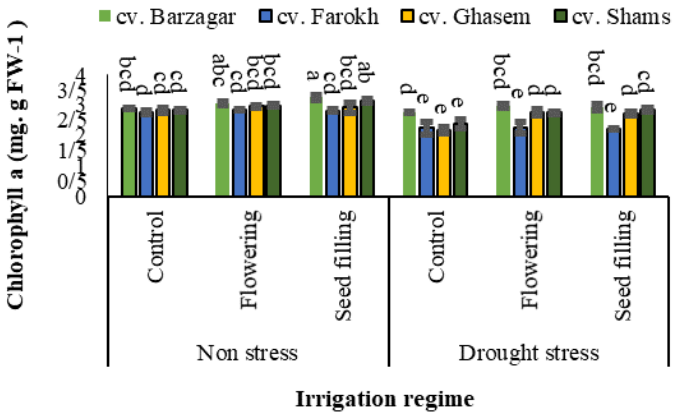

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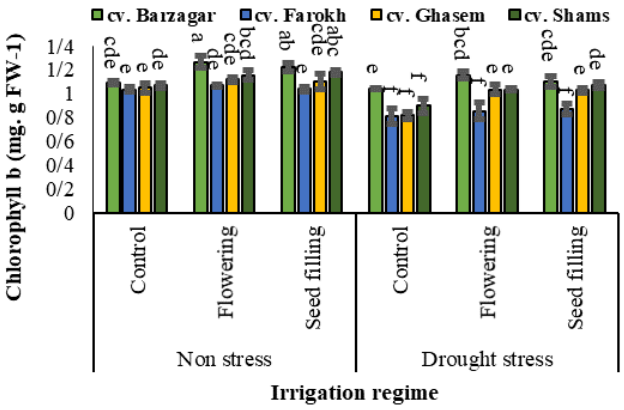

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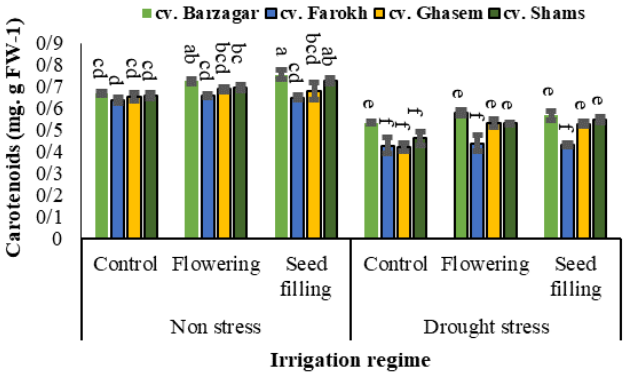

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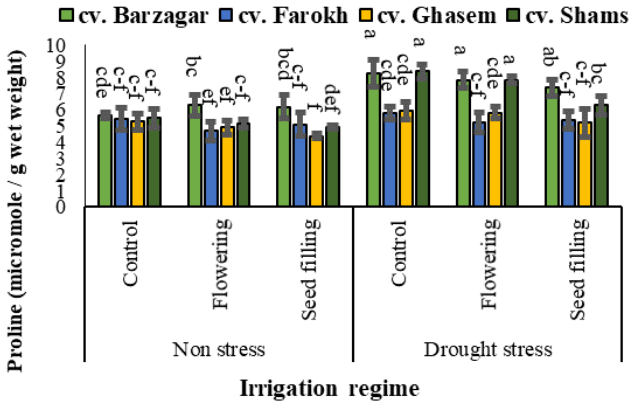
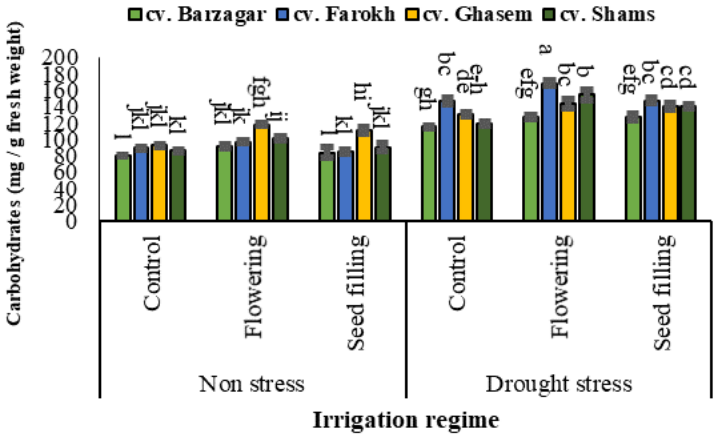
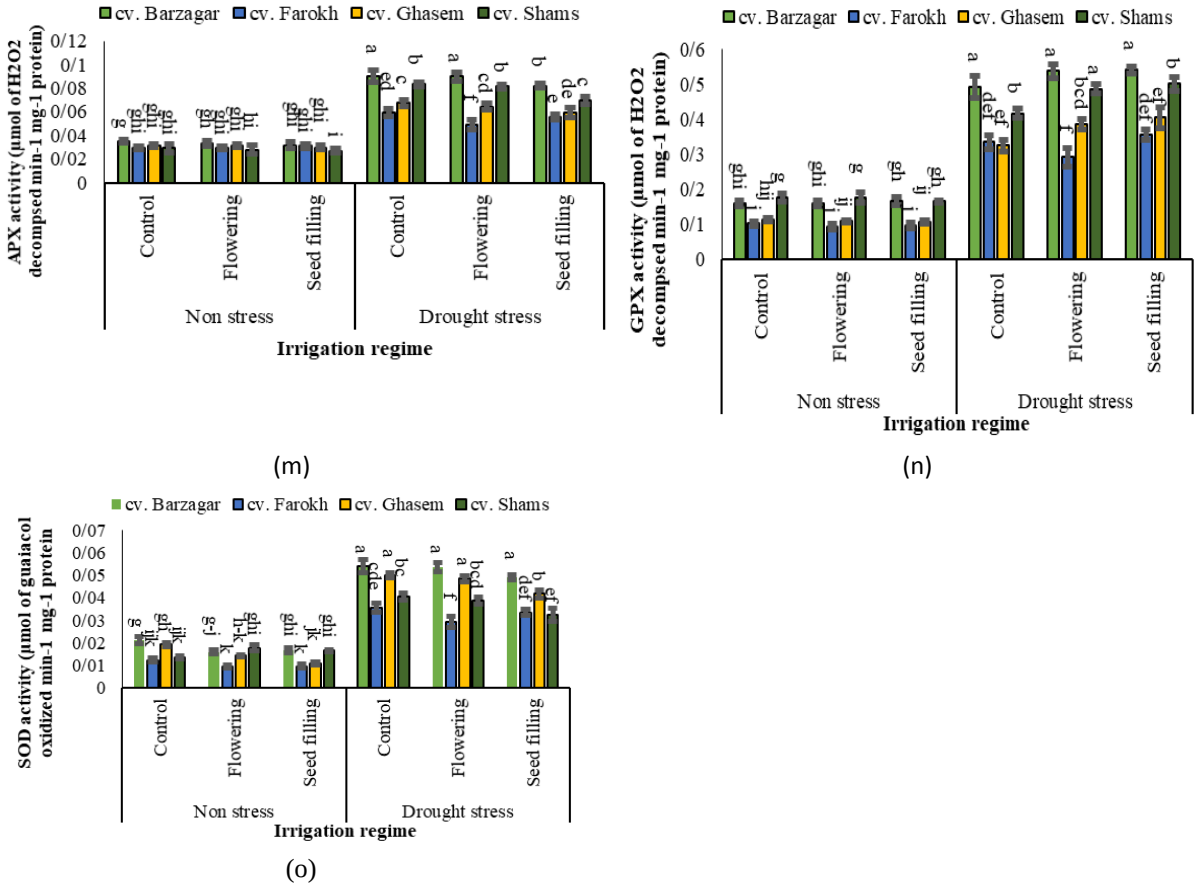


(k)



(l)

Continued of Figure 2. The effect of Nano-magnesium foliar application on seed yield (a), oil percentage (b), total protein (c), RWC (d), ELL (e), MDA (f), total chlorophyll (g), a chlorophyll (h), b chlorophyll (i), carotenoid (j), proline (k), soluble carbohydrates (l), APX (m), GPX (n), and SOD (p) activity in the four sunflower cultivars. Columns with at least one letter in common did not have a statistically significant difference based on the Duncan test at the 0.05 level. **Figure 2 Continued…**



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Altogether, cultivar Barzegar had the highest grain yield, oil percentage, and total protein content.

Based on our findings, Mg NPs spraying improved the leaf RWC under drought conditions in all cultivars. In NPs spraying at flowering and grain filling stages, the highest leaf RWC was recorded in cultivar Barzegar in drought (Figure 2-d). The leaf EL was significantly decreased by Mg NPs supply under drought. The NPs treatment declined the leaf EL of Barzegar cultivar by 1.36- and 1.3-fold in flowering and grain filling stages, respectively, as compared to the control (Figure 2-e). Mg NPs, in turn, partially decreased MDA content in all cultivars. In total, the lowest MDA content under drought stress was registered in Barzegar cultivar due to Mg NPs spraying at both spraying stages. The highest MDA content was recorded in the cultivar Ghasem (Figure 2-f).

NPs spraying significantly increased chlorophyll and carotenoid content in sunflower. In NPs spraying at flowering and grain filling stages, the lowest leaf chlorophyll and carotenoid content in stress was observed in Farrokh cultivar, while the highest level was found in Barzegar [Figure 2 (g-j)]. After NPs spraying, the highest leaf proline and soluble carbohydrates content

under drought was observed in Barzegar at the flowering stage (Figures 2-k and -l).

Mg NPs caused a slight increase in ascorbate peroxidase enzyme activity (Figure 2-m). After NPs treatment, glutathione peroxidase activity was highest in Barzegar and Shams cultivars under stress, whereas it was lowest in Farrokh and Ghasem cultivars (Figure 2-n). Moreover, superoxide dismutase activity was highest in Barzegar and Qasem cultivars under drought stress, while it was lowest in Farrokh and Shams cultivars (Figure 2-o).

DISCUSSION

Sunflower is an oil grain crop of subtropical and tropical areas with arid to semi-arid climate that is affected by unfavorable environmental factors such as drought. Drought at grain filling and flowering steps reduces significantly the grain yield in this oil crop (García-López *et al.*, 2014). Given the key role of Mg in many key metabolic processes (Cakmak and Yazici, 2010), it seems that Mg NPs foliar application can improve the sunflower tolerance to drought. There is no report on Mg NPs foliar application, although several studies have been conducted on Mg spraying. For instance, Howladar *et al.* (2014) observed Mg elevated significantly Ca/Na ratio, calcium, magnesium, potassium, phosphorus, nitrogen, free proline, soluble sugars, canopy dry weight plant^{-1}, total leaf area plant^{-1}, number of branches plant^{-1}, shoot length, and green pod and seed yields in *Pisum sativum*. Thus, the authors suggested that Mg has positive effects on pea plants. Singh *et al.* (2015) also reported the positive effect of Mg spraying on growth and yield of Bt cotton. Borowski and Michałek (2010) also showed Mg spraying led to an elevated leaf content of nitrates and protein in spinach. Thus, we evaluated the effect of Mg NPs on agronomical, physiological and biochemical traits under drought stress in sunflower.

As observed in this work, the grain yield and oil percentage in sunflower were slightly increased by Mg NPs treatment. Similarly, Jahangir *et al.* (2005) used various concentrations of magnesium (0, 7.5, 15 and 30 kg ha^{-1}) to *Brassica juncea* and assayed the effect on oil yield and quality. Although they observed yield increase due to Mg supply, this increment was not significant. However, the oil concentration was enhanced significantly from ~39 to ~41%. Researches on the effect of magnesium soil application (0–68 kg MgO ha^{-1}) on quality and yield of soybean revealed that increasing the Mg supply up to 40 kg MgO ha^{-1} enhanced yield significantly, whereas the oil level remained unaltered (Nelson *et al.*, 1945). It should be noted that Barzegar cultivar in sunflower as a late plant received more nano fertilizer and also had the number of grain per head and thousand grain weight. In this regard, it showed higher percentage of oil and yield among other cultivars. (Fatemi et al., 2021). Taken together, the findings showed that depending on the soil Mg availability, Mg NPs treatment can improve oil content and yield.

In the light of our findings, the Mg NPs foliar application improved leaf Relative Water Content (RWC) under normal irrigation and drought stress in all cultivars. The higher drought tolerance of the cultivars with high RWC are probably due to two factors. First, these cultivars keep their RWC at a higher level by closing their stomata and less transpiration under stress; and/or they have a strong root system and can absorb water from the soil depth and transfer it to the aerial parts, thus maintaining their RWC at high level (Sabzehzari and Naghavi, 2019a, b). In fact, cultivars having higher RWC are more resistant to drought due to the stable osmo-regulation (Hussain *et al.*, 2018; Gunasekera and Berkowitz, 1992). The stronger performance of the cultivars with high RWC has been proved in the current study; such that the high RWC Barzegar cultivar had the highest oil percentage and grain yield in drought. The leaf RWC improved by Mg NPs spraying under drought can be attributed to auxin signaling and stomata closure, although the exact mechanism is not cleared.

Mg NPs foliar application decreased leaf Electrolyte Leakage (EL) index and MDA content under drought stress in all cultivars that had been increased due to the reduced membrane fluidity and lipid peroxidation derived from ROSs activity. The low MDA and EL values in Barzegar cultivar may be the result of the activity of antioxidant enzymes, because these enzymes inactivate ROS and reduce their damage and,

consequently, cause membrane to become more stable (Hussain *et al.,* 2018). Cell membrane stability is one of the important resistance mechanisms to drought (Sabzehzari *et al.,* 2020a, b). Since drought leads to disturbance in the biological role of cell membrane, declines fluidity and ions pumping speed (Bajji *et al*., 2002), the lower EL is considered as a tolerance parameter. The stronger performance of the cultivars with lower MDA and EL under drought has been documented in our study; Barzegar cultivar with the lowest MDA and EL had the highest oil percentage and grain yield in drought. The leaf EL and MDA dropped by Mg NPs application in drought and can be attributed to Mg–mediated stronger antioxidant system, even though the exact underling mechanism is not clear.

Drought significantly decreased chlorophyll and carotenoid contents, whereas Mg NPs spraying significantly increased them. The capability to maintain the chlorophyll content of leaf under environmental stress was utilized as an index in resistant genotypes selection (Farooq *et al.,* 2009). Moreover, the carotenoid content of leaf plays a key role in drought tolerance (Norouzi *et al.,* 2015). High level of carotenoids in adverse environments can be a reason for plant resistance to stress because these pigments are one of the non-enzymatic antioxidants and can protect the plant against oxidative stress (Sabzehzari *et al*., 2019, 2020c; Farooq *et al.,* 2009). As a result, the higher content of these pigments has been characterized as a mechanism to alleviate the adverse efficacies of drought-induced photo-inhibition (Silva *et al.,* 2007). A plant that can keep its chlorophyll content high and has better photosynthesis ability, can withstand stresses better. Based on our observations, the higher leaf chlorophyll content can be due to the fact that Mg is a key element involved in the chlorophyll biosynthesis (Canavar *et al.,* 2014).

Drought stress significantly increased leaf proline content and Mg NPs foliar application caused a slight increase in leaf proline content. As reported previously, the increased proline content under drought may act as a cytoplasmic osmolyte in osmotic adjustment and maintain RWC to improve tolerance. Given the status of RWC and proline content in Barzegar cultivar, it can be concluded that the increased proline content in drought maintains hydration in plant cells and hampers damage. On the other hand, proline serves as an osmolyte and a storage resource for nitrogen and carbon, which are afterward utilized for stabilizing a variety of macromolecules such as proteins and for maintaining cell membranes in plant cells (George *et al.,* 2015; Serraj and Sinclair, 2002). The leaf proline content slightly changed in response to NPs supply in drought. In a study on soybean, Fang *et al.* (2004) stated that Mg application increased the content of protein and soluble sugar in leaf, while declining the free praline content lightly.

As our results revealed, drought significantly increased soluble carbohydrate content and Mg NPs foliar application caused a slight increase in them. Soluble sugars act as metabolic resources, regulate several processes involved in growth and development, operate as an osmo-protectant under water stresses (Snchez *et al.,* 1998), and help to maintain a healthy photosynthetic system and water balance in plants as described by Sinha *et al.* (2018). Notably, the increased level of soluble carbohydrates in Mg NPs-treated sunflower under drought can be due to the vital role of Mg in carbohydrate synthesis and activation of many metabolic enzymes (Rady and Osman, 2010). As observed in this study, Bogdevich and Mishuk (2006) demonstrated that Mg supply enhanced the carbohydrate content in *Brassica napus* only at a soil with low Mg.

The changes in expression and synthesis of proteins have been documented in many plants as a result of exposure to drought during growth and development (Bray, 1993). It appears that the primary increment in soluble proteins during stress is due to the expression of novel stress proteins; however, the subsequent decrement is due to a severe decline in the photosynthetic process (Kramer, 1974). Foliar application of Mg NPs increased the amount of total protein at flowering and grain filling stages. Similarly, Vrataric *et al.* (2006) recorded a significant increase in protein content upon Mg foliar treatment of 5% $MgSO_4 \cdot 7H_2O$ solution during vegetative growth. This increment can be explained by the fact that Mg has a key role in protein synthesis and amino acid transport (Cakmak and Yazici, 2010). Further, the active forms of ribosome require assembling of two subunits, involving Mg to generate a bridge

between the subunits. Therefore, protein biosynthesis is greatly affected by Mg level, resulting in changed concentration of the precursor amino acids (Cakmak and Yazici, 2010).

The higher level of CAT, APX and SOD activities under drought is proposed to be related to a higher level of tolerance by several authors (Huang *et al.,* 2013; Sofo *et al.,* 2015). In this experiment, drought stress significantly increased the activity of antioxidant enzyme, whereas Mg NPs foliar application caused a slight increase in stress level, this increment was highest in Barzegar and lowest in Farrokh. In line with our findings, da Silva *et al.* (2017) reported that the antioxidant metabolism is changed in coffee seedlings exposed to Mg level. However, Uzilday *et al.* (2017) stated that total activity of glutathione-S-transferase, glutathione reductase, peroxidase, APX, CAT, and SOD increased with Mg deficiency.

Based on the previous researches and our recent findings, it seems that the positive effect of MgNPs foliar application on the sunflower grain yield and oil percentage, physiological and biochemical traits under drought is due to the specific metabolic reactions and processes that are affected by Mg. These processes include photo-phosphorylation, chlorophyll generation, photo-oxidation in leaves tissue, utilization and partitioning of photo-assimilates, water potential adjustment, protein synthesis, photosynthetic CO_2 fixation, production of reactive oxygen species, and antioxidant system (Cakmak and Yazici, 2010; Canavar *et al.*, 2014). However, the exact mechanisms and processes underlying MgNPs- derived drought resistance in sunflower and other crops need further evaluation.

CONCLUSIONS

Our results revealed that Mg NPs compensate the sunflower production losses that happen when drought occurs at flowering and grain filling stages through the improved antioxidant system, enhanced photosynthetic pigments, and increased primary metabolites. Given our findings, MgNPs foliar application on sunflower seems to be helpful in overcoming the negative effects of drought stress and, eventually, in increasing grain yield and oil percentage.

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نانو ذرات منیزیم، صفات زراعی، مورفولوژیک و بیوشیمیایی آفتابگردان را تحت شرایط خشکی بهبود می‌بخشند

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چکیده

آفتابگردان (*Helianthus annuus* L.) یک گیاه دانه روغنی در مناطق نیمه گرمسیری و گرمسیری با آب و هوای خشک تا نیمه خشک است و تولید آن در مراحل پر شدن دانه و گلدهی تحت تأثیر خشکی قرار می‌گیرد. با توجه به نقش کلیدی منیزیم (Mg) در بسیاری از فرآیندهای متابولیکی، به نظر می‌رسد نانوذرات منیزیم بتوانند عملکرد آفتابگردان را در تنش خشکی بهبود بخشند. این آزمایش بصورت کرت‌های دوبار خردشده در قالب طرح بلوک کامل تصادفی با سه فاکتور شامل فاکتور اول: رژیم آبیاری (آبیاری نرمال و تنش خشکی)، فاکتور دوم: ارقام آفتابگردان (فرخ، شمس، قاسم و برزگر)، و فاکتور سوم: زمان کاربرد نانوذرات منیزیم (محلول‌پاشی در مرحله گلدهی و محلول-پاشی در مرحله پر شدن دانه) با ۳ تکرار در مزرعه در دو سال ۹۷-۱۳۹۶ و ۹۸-۱۳۹۷ انجام شد. چندین صفت فیزیکی-بیوشیمیایی همراه با عملکرد دانه و درصد روغن اندازه گیری شدند. بر اساس نتایج، محلول‌پاشی نانوذرات منیزیم باعث افزایش محتوای آب نسبی، کلروفیل و کاروتنوئید، کربوهیدرات-های محلول و فعالیت آنزیم‌های آنتی اکسیدانی تحت خشکی شد. علاوه بر این، محلول‌پاشی نانوذرات منیزیم باعث کاهش نشت الکترولیت و محتوای مالون دی آلدئید در گیاهان تحت تنش شد. کاربرد نانوذرات منیزیم در مراحل گلدهی و پر شدن دانه، عملکرد دانه و درصد روغن را به مقدار اندکی افزایش داد. به طور کلی، یافته‌های ما نشان داد که نانوذرات منیزیم با بکارگیری چندین مکانیسم از جمله سیستم آنتی‌اکسیدانی بهبود یافته، رنگدانه‌های فتوسنتزی بیشتر و سطح بالاتر متابولیت های اولیه، عملکرد آفتابگردان را در تنش خشکی بهبود می بخشند.