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Research Paper

Improving physiological performance and productivity of oilseed rape under drought stress by foliar application of Zn and Mg nanoparticles

Kazem Ghassemi-Golezani[1*], Mahdieh Rajabi[2], and Roghiyeh Farzi-Aminabad[3]

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[1]Professor, Department of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2]Master of Agronomy, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[3]PhD Student in Crop Physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
*Corresponding author; Email: golezani@gmail.com

Abstract
A field experiment was laid out as a split plot design based on the randomized complete block design with three replications in 2018, to assess the responses of oilseed rape (*Brassica napus* L.) plants to foliar treatment of nanoparticles (ZnO and MgO) under different watering levels (I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130, and 160 mm evaporation as normal irrigation, and mild, moderate, and severe stresses, respectively). Water shortage increased leaf temperature and decreased leaf water content, membrane stability, chlorophyll content, and plant biomass, which resulted in the reduction of the grain yield per unit area. Foliar application of nanoparticles enhanced grain and oil yields of oilseed rape by reducing some detrimental impacts of water limitation on leaf temperature, chlorophyll content, membrane stability, plant height, plant biomass, grains per plant, and 1000-grain weight. Therefore, foliar spray of these nanoparticles could be a superior treatment for alleviating some of the adverse effects of drought stress on the physiology and productivity of oilseed rape plants in the field.

Keywords: chlorophyll content, leaf temperature, membrane stability, oil content, oilseed rape

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Introduction

Oilseed rape (*Brassica napus* L.) is an annual crop with two winter and spring types that is cultivated under various environmental conditions. Although oilseed rape to some extent tolerates drought stress, severe water deficit could disrupt the physiological processes and productivity of this crop (Ghassemi-Golezani *et al*. 2019). Drought stress occurs when water availability is less than the critical level for plant growth and development (Boyer 1976).

Drought stress can influence different physiological processes in plants, leading to poor performance and yield (Ghassemi-Golezani and Mousavi 2022). The extent of drought impacts on crop growth and yield varies with species, growth stage, and severity of stress (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Water shortage could reduce the photosynthetic rate by closure of the stomata or by a reduction in chlorophyll content and photosynthetic capacity of the leaves (Ghassemi-Golezani *et al*. 2018). Stomatal closure also results in higher leaf temperature due to the reduction in transpiration cooling under water stress (Lu *et al*. 1997), leading to the combination of drought and heat stresses (Mohammadian *et al.* 2005). Drought stress severely limits plant growth and yield by reducing ground green cover (Ghassemi-Golezani *et al.* 2022), chlorophyll content of leaves, and photochemical efficiency of photosystem II (Ghassemi-Golezani *et al.* 2016). Most of the drought-tolerant crops have a high root/shoot ratio that can help to absorb more water from the soil and largely avoid from detrimental effects of this stress, due to high leaf water content. The high leaf water content is the result of higher osmotic regulation of plants under limited water availability (Ghassemi-Golezani *et al*. 2016). Drought stress can also limit nutrient uptake by the plants, which could be largely overcome by foliar spray of essential elements.

The foliar spray of essential elements could be an effective option when nutrient deficiencies cannot be compensated by soil fertilization (Ghassemi-Golezani *et al.* 2022). Since various factors influence nutrient uptake of plants in the field, foliar application of nutrients is more beneficial than soil fertilization (Khan *et al*. 2003). Foliar treatment of some nanoparticles may enhance the tolerance of plants to stressful conditions such as drought (Ghassemi-Golezani and Afkhami 2018) and salinity (Weisany *et al*. 2011; Ghassemi-Golezani and Abdoli 2021). Application of nanoparticles at low rates increases nutrient uptake and nutrient use efficiency and decreases losses through leaching and gaseous emissions along with reducing the risk of nutrient toxicity for ensuring food security (Iqbal 2019). According to Freitas *et al.* (2021), there is room for more research on the effects of nanoparticles in mediating plant stress responses for future agricultural research developments. The nanoparticles can promote plant growth by facilitating efficient input delivery networks and enriching plant health (Chugh *et al.* 2021).

Zinc has been considered an essential micronutrient for metabolic activities in plants including enzyme activities and biochemical reactions responsible for chlorophyll and carbohydrate formations (Auld 2001; Ghassemi-Golezani *et al.* 2022). Magnesium oxide (MgO) nanoparticles which are non-toxic and cheaply available, may have important roles in the morpho-physiological functioning of the plants (Shen *et al.* 2020). Photosynthetic attributes along with chlorophyll content have been reported to increase significantly in plants treated with MgO nanoparticles (Raliya *et al.* 2014). However, the effects of Zn and Mg nanoparticles on the field performance of some plants under drought stress are not clear. So, the objective of this research was to evaluate the responses of oilseed rape (*Brassica napus* L.) plants to foliar spray of nanoscale zinc-oxide and magnesium-oxide under normal and limited irrigation intervals.

Materials and Methods

A field experiment with a split plot arrangement based on the randomized complete block design in three replications was undertaken in 2018 to investigate the impacts of foliar applications of zinc and magnesium nano-particles on oilseed rape (*Brassica napus* L.) performance under various levels of water supply. The experimental plots were located in the Research Station of the University of Tabriz, Tabriz, Iran (Latitude 38° 05´N, longitude 46° 17´E, altitude 1360 m above sea level), with a mean annual precipitation of 285 mm and mean annual temperature of 10 °C. The properties of soil in the 0-30 cm depth are presented in Table 1.

Irrigation treatments (I_1, I_2, I_3, and I_4: irrigation after 70, 100, 130, and 160 mm evaporation from class A pan as normal irrigation and mild, moderate, and severe water deficits, respectively) and foliar sprays (water, 1g/l nano-ZnO, and 1g/l nano- MgO) were assigned to the main and subplots, respectively. Six rows with a 2 m length and 25 cm distance within each plot were prepared for sowing. The purity of nanoparticles was +99%, and the particle sizes of nano-ZnO and nano-MgO were 10-30 nm and 20 nm, respectively.

Seeds of cultivar Dalgan (spring oilseed rape) were provided by the Seed and Plant Improvement Institute, Karaj, Iran. The seeds were pretreated with 2 g/kg benomyl and then were sown by hand in the 1-2 cm depth of soil with a density of 80 seeds/m^2. All plots were regularly irrigated up to crop establishment, but subsequent irrigations were carried out according to the irrigation treatments. Weeds were controlled by hand as required. Water and nanoparticles were sprayed on plants at the flowering stage. Physiological traits were measured at the pod-filling stage, but individual plant biomass and grain yield were determined at maturity.

Table 1. The properties of experimental soil in 0-30 cm depth

Depth (cm)	pH	Total neutralizing material (%)	Electrical conductivity (dS/m)	Total nitrogen (%)	Organic carbon (%)	Absorbable elements (mg/L)						Mineral components of soil (%)			Soil texture
						K	P	Fe	Ca	Mn	Zn	Silt	Clay	Sand	
0-30	8.0	10.5	2.92	0.04	0.37	255	4.9	2.6	0.76	3.34	0.92	14	12	74	Sandy loam

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Physiological traits

Leaves of a random plant from each plot were separated and cut into small pieces. Then, 10 g of fresh leaf discs were weighed, dried in an oven for 48 h at 75 °C, and reweighed. Leaf water content (LWC) was determined as:

$$LWC\ (\%) = ((FW - DW) / FW) \times 100$$

where FW is the leaf fresh weight and DW is the dry weight.

Leaf temperature (°C) was measured by an infrared thermometer (TES-1327) in the upper, middle, and lower leaves of each plant from each plot just before irrigation at the pod-filling stage. Then, the mean temperature was calculated for each plot.

The membrane stability index (MSI) was determined by recording the electrical conductivity of leaf leachates in the double distilled water at 40 °C and 100 °C. Leaf samples (0.5 g) were washed and cut into five discs of uniform sizes and taken in the test tubes containing 10 ml of distilled water in two sets. One set was kept at 40 °C for 30 min and another set at 100 °C for 10 min. The electrical conductivities were recorded by a conductivity meter (LS90, WTW GmbH, Germany), and the MSI was calculated as:

$$MSI = (EC40°C / EC100°C) \times 100$$

The chlorophyll content index (CCI) was measured by a chlorophyll meter (CCM-200, Opti- Science, USA) in the upper, middle, and lower leaves of each plant from each plot. Then, the mean CCI was calculated for each plot.

Plant biomass and yield components

At maturity, 10 competitive plants were harvested from each plot, and plant height, grains per pod, grains per plant, 1000-grain weight, and grain yield per unit area were determined. Subsequently, these plants were dried in an oven at 75 °C for 48 hours and plant biomass for each plot was determined.

Oil content

The oil content of grains was extracted by a Soxhlet, using the method by AOCS (1993). Three grams of the oilseed rape grains from each plot were powdered by a milling machine. The samples were poured into filter-paper tubes and placed in the Soxhlet extractor. The sum of 200 ml petroleum ether and a few pieces of stone were poured into a flask. The Soxhlet extractor and refrigerant were then installed on the flask. Then, the faucet was turned on to allow water to flow into the refrigerant. When the first drop of solvent was distilled and dripped from the refrigerant, the time was recorded. The extraction of oil was continued for five hours and the heat source was then turned off, cooling the vapor into a liquid. The flask was then removed from the clamp and the samples were weighed. Finally, the oil percentage and yield were determined.

Statistical analysis

Analysis of variance appropriate to the experimental design was conducted, using SAS 9.4 and MSTAT-C software. The means of each trait were compared by the Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

Results and Discussion

Analysis of the resulting data revealed that individual effects of irrigation levels and nanoparticles and also the interaction of these factors were significant for leaf temperature, CCI, MSI, plant biomass (Table 2), grains per pod, grains per plant, grain yield per unit area, oil percentage, and oil yield (Table 3). The plant height (Table 2) and 1000-grain weight were only affected by the water limitation and foliar treatment (Table 3). However, LWC was only affected by irrigation levels (Table 2).

Leaf water content

LWC was considerably reduced under severe water deficit (I_4), but LWC reduction under mild (I_2) and moderate (I_3) water limitations was not statistically significant compared to the normal irrigation (Figure 1A). There is a direct relationship between LWC and drought tolerance (Dastborhan and Ghassemi-Golezani 2015). According to Liu *et al.* (2002), the reduction of LWC under water deficit was associated with decreasing plant growth and vigor A small, but not significant increase in LWC of nanoparticles-treated plants (Figure 1B) may have been achieved by increasing the root growth (Prasad *et al.* 2012; Raliya *et al.* 2014).

Table 2. Analysis of variance of the effects of foliar sprays of nano-ZnO and nano-MgO on some morpho- physiological traits of oilseed rape under different irrigation intervals.

Source	df	Leaf water content	Leaf temperature	Membrane stability Index	Chlorophyll content index	Plant height	Plant biomass
Block	2	8.22	0.098	2.69	10.02	14.74*	0.09
Irrigation (I)	3	151.47**	108.239**	2023.89**	359.39**	70.44**	7.11**
Error a	6	16.34	0.211	3.58	19.59	10.21	0.03
Nanoparticle (NF)	2	21.09	7.427*	1680.03**	2056.87**	34.63**	18.23**
I×NF	6	13.08	0.94037*	334.69**	53.22**	2.71	0.559**
Error b	16	18.32	0.421	12.36	17.35	3.21	0.139
CV (%)	-	3.35	2.895	2.49	4.58	3.49	4.67

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; CV: Coefficient of variation.

Table 3. Analysis of variance of the effects of foliar sprays of nano-ZnO and nano-MgO on yield components and grain yield of oilseed rape.

Source	df	Grains per pod	Grains per plant	1000-grain weight	Grain yield	Oil content	Oil yield
Block	2	0.46	455.75	0.01	13.16	9.78	4.7
Irrigation (I)	3	21.92**	66477.46**	2.04**	7320.34**	247.93**	844.14**
Ea	6	0.44	955.42	0.08	5.97	3.96	0.99
Nanoparticle (NF)	2	7.23**	24745.89**	0.58**	246.51**	86.09^{ns}	340.59**
I×NF	6	2.73**	4541.9**	0.11^{ns}	246.51**	10.53*	28.52**
Eb	16	0.64	504.05	0.04	6.7	2.7	1.87
CV (%)	-	12.57	10.04	6.52	4.43	6.7	8.75

*, **: Significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; CV: Coefficient of variation.

Leaf temperature

Leaf temperature was generally increased with increasing the water deficit. The leaf temperature of nanoparticles-treated plants was almost lower than untreated plants, particularly under moderate and severe drought stresses (Table 4). Variation in leaf temperature due to water stress and nanoparticle treatments is directly related to changes in LWC. So, decreasing water availability under drought stress increased the leaf temperature, but foliar application of nanoparticles on plants decreased leaf temperature via increasing (although not significantly) the water status of the leaves (Figure 1). The rate of water uptake under drought stress cannot match the transpiration rate and stomata close to maintaining the water balance of plants. This can enhance leaf temperature even higher than the air temperature (Larcher 2000).

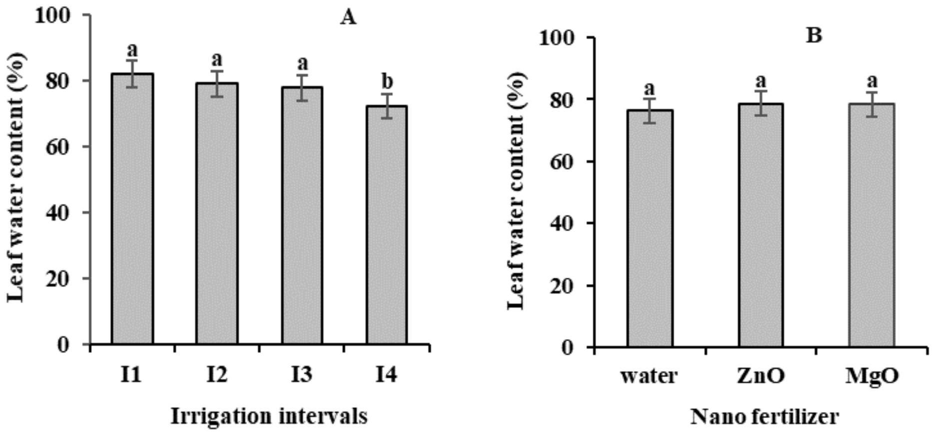

Figure 1. The oilseed rape leaf water content under different irrigation levels (A) and nano-fertilizers (B); I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130, and 160 mm evaporation, respectively; Different letters indicate a significant difference at $p \leq 0.05$.

Membrane stability index (MSI)

The MSI of the untreated plants was significantly reduced under moderate and severe water deficits. The MSI of nano-ZnO-treated plants was the highest under I_1, I_2, and I_4 watering levels, but the foliar spray of nano-MgO was the superior treatment under moderate (I_3) water deficit (Table 4). Reduction in MSI can be attributed to higher leaf temperature (Table 4) as a result of lower LWC (Figure 1A). Enhancing MSI of nanoparticle-treated plants could be also related to increasing LWC (Figure 1B) and antioxidant capacity for reducing oxidative damage of membranes and ion leakage (Ducic and Polle 2005).

Chlorophyll content index (CCI)

The CCI of the plants treated with nanoparticles, especially by nano-MgO was higher than that of the untreated plants at all levels of irrigation, particularly under moderate and severe stresses (Table 4). Chlorophyll is the main photosynthetic pigment in plants. The chlorophyll content somewhat reflects the rate of photosynthesis in plants, which is strongly affected by environmental factors (Qiu *et al*. 2007). Decreasing CCI under drought stress was associated with decreasing LWC (Figure 1A) and increasing leaf temperature (Table 4), leading to an increment in chlorophyllase activity (Reddy and Vora 1986). Enhancing CCI by nano-MgO-treatment was related to the role of Mg in the chlorophyll structure (Cakmak and Yazici 2010).

Plant height

Increasing leaf temperature and decreasing chlorophyll content index due to water deficit (Table 4) led to a significant reduction in plant height of the oilseed rape (Figure 2A). Similar results were reported for safflower (Ghassemi-Golezani and Afkhami 2018). The MgO nanoparticles increased plant height, but there was no significant difference between ZnO-treated and untreated plants (Figure 2B). Magnesium increases shoot growth and plant height through increasing CCI (Table 4) and photosynthesis.

Plant biomass

Plant biomass decreased as the water supply diminished. Foliar treatment of nanoparticles significantly enhanced plant biomass under all irrigation intervals. The nano-MgO was the superior treatment followed by nano-ZnO. Reduction in plant biomass, especially under moderate and severe drought stress was the result of increased leaf temperature and decreased CCI (Table 4), leaf area expansion, and plant growth (Ghassemi-Golezani *et al*. 2009). Enhancing plant biomass by nanoparticle treatments was related to the changes in leaf temperature and CCI (Table 4). Magnesium plays an important role in the synthesis of chlorophyll and has a key role in several physiological processes such as the loading of photosynthates from leaves to the sinks, thereby improving plant biomass (Verbruggen and Hermans 2013). Zn also has a positive role in the formation of chlorophyll and carbohydrates, leading to an increase in plant biomass (Corredor *et al*. 2009).

Grains per pod and grains per plant

Water stress reduced grains per pod and per plant, but foliar application of nanoparticles significantly enhanced these traits. Ghassemi-Golezani *et al.* (2018) reported that the main reason for a decline in the number of grains per plant under drought stress is the reduction in plant biomass. Foliar application of nano-MgO was the superior treatment for enhancing grains per pod and per plant under various levels of irrigation. These changes in the number of grains per pod and per plant are directly related to variations in chlorophyll content under different treatments (Table 4), that influenced photosynthesis and pod filling. Increasing grains per pod of the oilseed rape by the MgO treatment was also supported by Sikorska *et al.* (2020).

1000- grain weight

Decreasing water availability reduced 1000-grain weight, with no significant difference between normal irrigation and mild stress (Figure 3A). Water shortage reduces grain weight and yield by shortening the grain filling duration (Gooding *et al.* 2003). Foliar application of MgO and ZnO nanoparticles

Table 4. The means of some traits for different irrigation levels and foliar sprays in the oilseed rape.

Irrigation treatment	Nanoparticle	Leaf temperature (°C)	Membrane stability (%)	Chlorophyll content index	Plant biomass (g/m^2)	Grains per pod	Grains per plant	Grain yield (g/m^2)	Oil (%)	Oil yield (g/m^2)
	Water	16.99ef	59.67d	56.83ef	4.49e	8.17ab	248.43c	67.07e	27.88bc	18.7d
I_1	ZnO	15.59g	85.67a	59.48de	6.66b	6.08cd	298.31b	84.53c	28.8bc	24.37c
	MgO	15.83g	64.67c	71.32b	7.76a	8.87a	371.91a	106.48a	31.92a	33.98a
	Water	17.49de	58.33d	53.95fg	4.33e	6.31cd	214.98c	57.62f	25.7c	14.81e
I_2	ZnO	17.83de	84.67a	62.29cd	5.01d	7.06bc	248.04c	75.25d	26.33bc	19.79d
	MgO	16.16fg	64.00c	79.20a	6.96b	9.16a	384.23a	101.71b	29.35ab	29.83b
	Water	20.01c	44.33e	44.33h	3.41f	5.3de	152.89e	32.49h	19.11d	6.21g
I_3	ZnO	18.05d	69.00b	57.54def	4.21e	5.7de	170.24de	40.29g	26.65bc	10.77f
	MgO	18.12d	86.33a	77.18a	5.70c	6.62cd	210.43cd	58.63f	26.48bc	15.59e
	Water	25.00a	30.67g	35.63i	3.67f	4.57ef	129.93e	23.8i	15.14e	3.59h
I_4	ZnO	23.51b	47.00e	49.66g	4.36e	4.24f	123.84e	25.35i	15.63e	3.94gh
	MgO	23.32b	38.33f	66.61bc	5.36cd	4.32ef	130.62e	27.82i	21.47d	6.04g

Different letters in each column indicate significant differences at $p \leq 0.05$; I1, I2, I3, and I4: Irrigation after 70, 100, 130, and 160 mm evaporation, respectively.

A

B

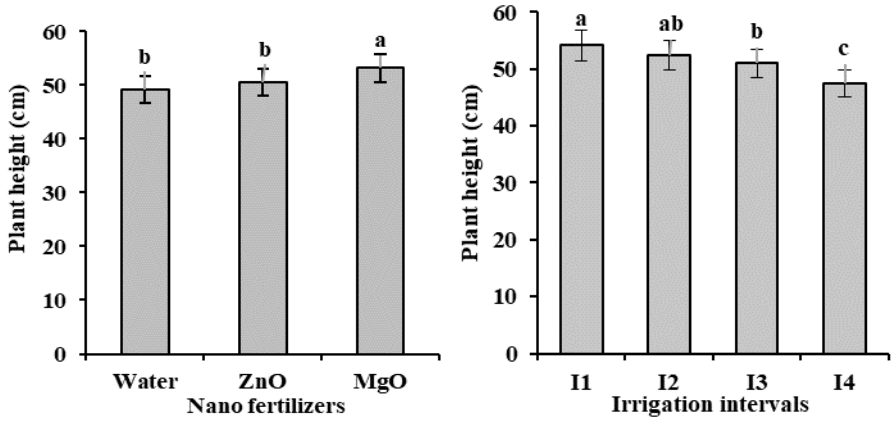

Figure 2. The oilseed rape plant height under different irrigation levels (A) and nano fertilizers (B); I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130, and 160 mm evaporation, respectively; Different letters indicate a significant difference at $p \leq 0.05$.

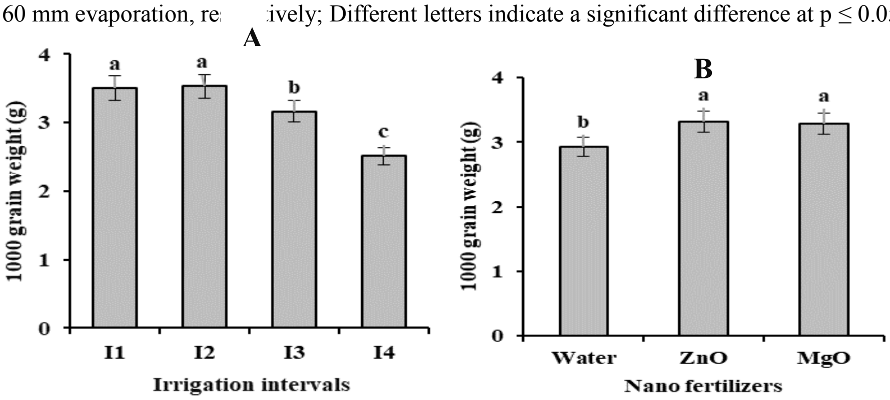

Figure 3. The oilseed rape 1000-grain weight under different irrigation levels (A) and nano fertilizers (B); I_1, I_2, I_3, I_4: Irrigation after 70, 100, 130, and 160 mm evaporation, respectively; Different letters indicate a significant difference at $p \leq 0.05$.

increased 1000-grain weight (Figure 3B). Khan and Khan (2015) also reported that foliar application of ZnO increased the 1000-grain weight of maize. Higher grain weight in the ZnO-applied plots could be attributed to the micro-element effects on the leaf area development, and greater assimilate production (SeifiNadergholi *et al*. 2011).

Grain yield

The grain yield per unit area was significantly decreased by drought stress, but it was increased by foliar treatments of ZnO and MgO in all watering levels. The higher grain yield was recorded in the normal irrigation with the MgO treatment, with no difference between normal irrigation and the mild water deficit (Table 4). Loss of grain yield, especially under moderate and severe drought stress was the result of decreasing CCI, plant biomass, grains per pod, and grains per plant (Table 4). The ZnO nanoparticles are stable and affordable to synthesize and have shown an appreciable potential to improve crop productivity under abiotic stresses such as drought (Deka *et al.* 2019). Fatemi *et al.* (2022) also reported that MgO nanoparticles compensated for the sunflower production losses under water stress, through the improved antioxidant system, enhanced photosynthetic pigments, and increased primary metabolites. The increased grain yield in response to Zn application could be due to the higher enzymatic activity that effectively increased photosynthesis and translocation of assimilates to the grains (Zayed *et al.* 2011).

Oil content

Foliar application of ZnO and MgO increased the oil content of oilseed rape in all irrigation levels. The highest oil percentage and oil yield were obtained in the MgO-treated plants under normal irrigation, with no significant difference between the normal irrigation and mild stress. Decreasing the oil yield of oilseed rape with increasing water shortage was the consequence of decreasing grain yield and oil percentage (Table 4). The low oil content of drought-stressed plants may be the result of a shorter grain-filling period under stress (Farzi-Aminabad *et al.* 2021). This stress-induced reduction of oil content could be also related to the grain's capacity for oil accumulation and oxidation (Mohammadi *et al.* 2018). The higher oil production values may be related to MgO and ZnO abilities to stimulate enzymatic activities (Nandhini *et al.* 2019).

Conclusions

Moderate and severe water deficits reduced grain yield and oil content of oilseed rape through increasing leaf temperature and decreasing LWC, MSI, CCI, and plant biomass. However, foliar spray of nano-ZnO and nano-MgO improved yield components and grain and oil yields, mainly through enhancing MSI, CCI, and plant biomass under normal and limited irrigations. Therefore, foliar application of MgO and ZnO nanoparticles could be an effective way to improve the physiological performance and productivity of oilseed rape plants 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 عملکرد فیزیولوژیک و تولید کلزا تحت تنش خشکی با محلول پاشی نانو ذرات روی و منیزیم

کاظم قاسمی گلعذانی [1*]، مهدیه رجبی[2] و رقیه فرضی امین آباد[3]

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

۲- کارشناسی ارشد زراعت، دانشکده کشاورزی، دانشگاه تبریز، تبریز

۳- دانشجوی دکتری فیزیولوژی گیاهان زراعی، دانشکده کشاورزی، دانشگاه تبریز، تبریز

مسئول مکاتبه؛ Email: golezani@gmail.com

چکیده

یک آزمایش مزرعه‌ای به صورت کرت‌های خرد شده در قالب طرح بلوک‌های کامل تصادفی با سه تکرار در سال ۱۳۹۷ ترتیب داده شد تا پاسخ کلزا (*Brassica napus* L.) به محلول پاشی نانو ذرات منیزیم و روی در سطوح مختلف آبیاری (I_1، I_2، I_3، I_4: به ترتیب آبیاری پس از ۷۰، ۱۰۰، ۱۳۰ و ۱۶۰ میلی متر تبخیر برای آبیاری معمول و تنش‌های ملایم، متوسط و شدید) ارزیابی گردد. کمبود آب دمای برگ را افزایش و محتوای آب برگ، پایداری غشا، محتوای کلروفیل و زیست توده گیاهی را کاهش داد که منجر به افت محصول دانه در واحد سطح گردید. محلول پاشی

نانوذرات از طریق کاهش برخی از اثرات مضر کمبود آب بر دمای برگ، محتوای کلروفیل، پایداری غشا، ارتفاع بوته، زیست توده گیاه، تعداد دانه در بوته و وزن هزار دانه، موجب افزایش عملکرد دانه و روغن کلزا شد. بنابراین، محلول پاشی این نانوذرات می‌تواند یک تیمار برتر برای کاهش برخی از اثرات نامطلوب تنش خشکی بر عملکرد فیزیولوژیک و تولید کلزا در شرایط مزرعه باشد.

واژه‌های کلیدی: پایداری غشا، دمای برگ، کلزا، محتوای روغن، محتوای کلروفیل