

## Foliar application of chitosan and nano-magnesium fertilizers influence on seed yield, oil content, photosynthetic pigments, antioxidant enzyme activities of sesame (*Sesamum indicum* L.) under water-limited conditions

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### Abstract

Applying elicitors and nano-fertilizer has been recommended to enhance the growth and yield of secondary metabolites in herbs and medicinal plants under water-limited stress. However, less information is available on the effects of chitosan and nano-magnesium fertilizers on sesame seed yield, oil content, and physiological traits in the presence of nano-magnesium chelate (nano-Mg) under water-limited supply. In this regard, field experiments as a split-factorial experiment was performed based on randomized blocks in three replicates in Varamin city, south of Tehran, Iran, during 2015-2016 to evaluate the impact of chitosan and nano-Mg on physiological, seed, and oil traits of sesame. Irrigation cut-off based on BBCH scale was considered as the main factor including normal irrigation (I<sub>1</sub>), irrigation cut-off in 75 (I<sub>2</sub>), and 65 BBCH (I<sub>3</sub>) stages. Secondary factors as the subplot included 'Oltan' (C<sub>1</sub>) and 'Dashtestan-2' (C<sub>2</sub>) sesame cultivars, and foliar application of nano-Mg (application and non-application) and chitosan (control (CH<sub>1</sub>), foliar application of 4.8 g L<sup>-1</sup> in 65 BBCH (CH<sub>2</sub>), and 6.4 g L<sup>-1</sup> in 75 BBCH stages (CH<sub>3</sub>)). Further, free proline content, total sugars, the activity of antioxidant enzymes including catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1), and ascorbate peroxidase (APX, EC 1.11.1.11), photosynthetic pigments content, seed yield, and oil content were evaluated in the next stage. Based on the results, irrigation cut-off in 65 BBCH stage (flowering) significantly increased free proline content, total sugars, and the activity of antioxidant enzymes, CAT, POD, and APX. However, chlorophyll a, b, and total contents, seed yield, oil percentage, and yield decreased under water stress. 'Dashtestan-2' cultivar had the highest seed yield and oil content, and more tolerant cultivar under water-limited stress. Interestingly, the production of proline content and total sugars increased while the activity of antioxidant enzymes, CAT, POD, and APX decreased under application of nano-Mg and CH<sub>2</sub>, which influenced both sesame response and seed attributes. As a result, the production of some physiological traits in sesame cultivars may be regulated by adjusting the irrigating practices. Finally, the co-application of nano-Mg and CH<sub>2</sub> increased the seed yield and oil content of sesame under limited water supply in the arid and semi-arid region.

**Keywords:** chlorophyll; catalase; drought stress; elicitors; free proline

**Abbreviations:** APX: ascorbate peroxidase; CAT: catalase; nano-Mg: nano-magnesium chelate; POD; peroxidase

## Introduction

Sesame (*Sesamum indicum* L.) is annual and self-pollinating, which belongs to Pedaliaceae family. In addition, it is regarded as one of the oldest oysters, which can be adapted to warm and semi-warm regions, known as the queen of oilseed plants. The seeds of this plant contain 44-58% oils, 18-25% proteins, and 13.5% ~ carbohydrates. The proper quality of sesame including cholesterol level and the presence of some antioxidants such as Sameen, Samolin, and Samol play an important role in human health. On the other hand, it is considered as a tolerant plant to the drought stress (Roul *et al.*, 2017).

Water-limited stress is the major abiotic stress mainly in the agricultural fields all over the world, which affects almost all agricultural crops and diminishes the productivity considerably. Two-thirds of food production in the world is obtained through agriculture in drought stress conditions while more than 45% of agricultural land is permanently under drought stress (Yanni *et al.*, 2016). Therefore, sesame is a desirable plant for cultivation under drought stress conditions. In addition, most of the plant species respond to abiotic stress through molecular, biochemical and physiological modifications, and ultimately morphological adaptations (Taieb *et al.*, 2016). Further, plants have evolved many different mechanisms to deal with the occurrence of water-limited conditions (Kebbas *et al.*, 2018). The results of some research indicated that the drought stress generally results in destructing the chloroplasts, and decreasing chlorophyll content and the activity of enzymes in the Calvin cycle during the photosynthesis process (Anaya *et al.*, 2018). In this regard, Mafakheri *et al.* (2010) reported a decrease in leaf chlorophyll content due to drought stress. According to Farouk and Amany (2012), the toleration of the plants to the drought stress is achieved by various methods such as closing the stomata, osmotic regulation, and accumulation of compatible solution materials. When plants are exposed to drought stress, and osmotic stress in many osmotic adjustment processes results in accumulating non-toxic soluble materials such as proline and sugar in the cell. Further, the dehydration leads to oxidative stress, a process in which reactive oxygen species (ROS) react with lipids, proteins, nucleic acids, and cellular enzymes, which results in cell death. ROS is regarded as the result of electron transfer in mitochondria, chloroplasts, and cytosols, leading to the formation of superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), radical hydroxyl, and singlet oxygen. Further, the first defensive barrier against enzymatic and non-enzymatic antioxidants is the resistance toward ROS in plant cells. The first defence line against ROS to enzymatic and non-enzymatic antioxidants increases in plant cells. In this regard, the increased activity of antioxidant enzymes due to drought stress was reported by Murshed *et al.* (2013).

Water-limited stress leads to an imbalance in absorbing the nutrients from the soil and plant nutrition is very important in such a condition. Magnesium (Mg) has been implicated as a common ingredient in several critical processes of physiology and biochemical activity in plants including the formation of ATP in chloroplasts,  $CO_2$  stabilization, protein synthesis, chlorophyll formation, pharyngeal development, and optical oxidation in leaves. Furthermore, it is important for Grana accumulation in chloroplasts (Ceppi *et al.*, 2012). According to Senbayram *et al.* (2015), the deficiency of  $Mg^{2+}$  leads to plant damage, which is assumed that the availability of sufficient Mg is effective to achieve optimal performance in drought stress conditions. The use of Mg increases soluble sugars and protein in soybean. The deficiency of  $Mg^{2+}$  may cause oxidative stress inside plant cells. Under Mg deficiency condition,  $CO_2$  stabilization results in damaging the RUBP carboxylase by reducing the biosynthesis, which may lead to the production of ROS, damage to photosystem I and II, and a reduction in growth and yield of the plant. The results of some studies indicated a reduction in the concentration of soluble sugars and protein in soybean under Mg deficiency, a decrease in proline content, and an increase in the activity of catalase enzyme in rice (Chou *et al.*, 2010).

Chitosan ( $C_{11}H_{17}O_7N_2$ ) is a polyacetate polysaccharide, which is produced as a natural polymer from Alkaline N-deacetylation of chitin and is regarded as the second most abundant polymer on earth, which is the cell wall of some fungi and insects, leading to the production of some algae (Solgi, 2018). Further, this material created from the compounds with the properties of opiatetrium which impose plant defence mechanisms

against stress and improve plant growth. Some researchers believe that this substance can increase the activity of antioxidant enzymes and act as a neutralizing ROS (Sheikha and Al-Malki, 2011). Regarding the application of chitosan foliar, some studies reported an increase in chlorophyll content, catalase activity, soluble protein content, and soluble sugars and proline content (Pongprayoon *et al.*, 2013; Sultana *et al.*, 2017).

Nano-fertilizers include the nutrients with the size 30 to 40 nanometer and can carry nutrients properly due to their high surface. The use of nano-fertilizers to control the release of nutrients accurately can be considered as an effective step toward achieving sustainable and eco-friendly agriculture. Further, Mg is an important task for plants based on quantitative and qualitative, energy conservation, protein synthesis, structure stability, and sugar accumulation, as well as controlling pH in the cell and a cofactor for many enzymes (Blasco *et al.*, 2015).

Assessing the role of nano-fertilizers and elicitor as a strategy is important for reducing the negative effects of drought stress on plants. In this regard, the present study aimed to evaluate the effect of magnesium nano-chelate and chitosan elicitor on the activity of antioxidant enzymes, photosynthetic pigments, seed yield, and oil content of sesame under water-limited stress in order to provide a suitable way for reducing drought stresses and achieving sustainable agriculture in arid and semi-arid regions.

## Materials and Methods

### *Plant material and growth condition*

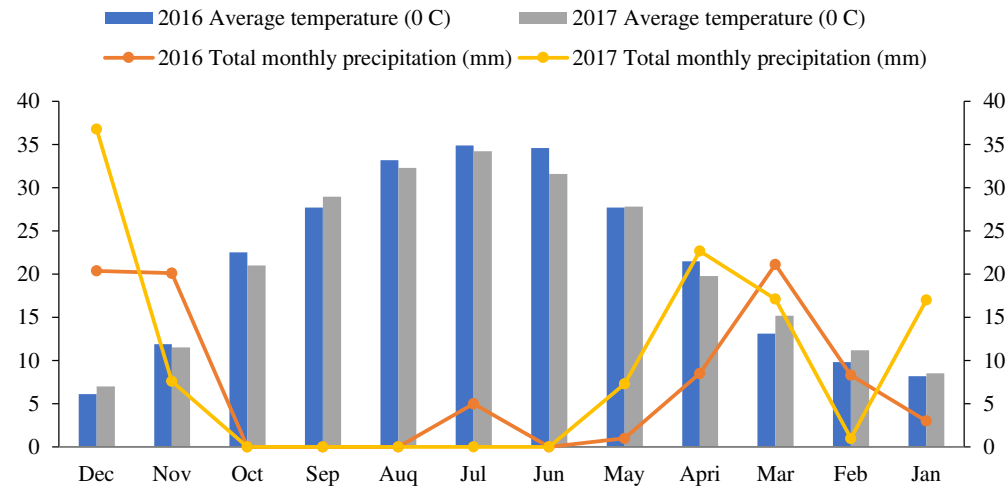
A field experiment was conducted to investigate the effects of using chitosan and nano-Mg fertilizers on some physiological traits of two sesame cultivars ('Oltan' and 'Dashtestan-2') in the second cultivation during 2015-2016. The experiment was performed in a research farm in Varamin city, the South of Tehran Province, Iran (35° 30' latitudes and 51° 73' longitudes) (Figure 1). Climate and meteorological data during the experiment period was shown in Figure 2. The physical and chemical properties of soil were analysed by using the standard methods (Table 1).

**Table 1.** The physical and chemical properties of the experimental fields (2015 and 2016)

Depth	pH	EC	OC	TNV	N	P	K	Mg	clay	silt	sand	Texture
cm	-	dS m <sup>-1</sup>	%	%	%	ppm	ppm	ppm	%	%	%	-
<b>2015</b>												
0-30	7.68	5.38	0.55	20.58	0.05	12	284.2	1.95	19	46	35	loam
<b>2016</b>												
0-30	7.61	4.96	0.56	20.46	0.04	13.5	245.3	1.71	20	44	36	loam



**Figure 1.** Sesame seedlings and plants in the research field during the experiments



**Figure 2.** Climate and meteorological data during the experiment period

*Experimental design*

A factorial split plot experiment was conducted based on a completely randomized block design (CRBD) with three replicates by using irrigation levels including control (normal irrigation: I<sub>1</sub>), irrigation up to seed ripening (75 BBCH=mild stress: I<sub>2</sub>), and irrigation up to flowering (65 BBCH=severe stress: I<sub>3</sub>) as the main plots. The foliar application of nano-Mg at 0 and 2 g L<sup>-1</sup> concentrations, chitosan at 0 (CH1), 4.8 (CH2), and 6.4 (CH3) g L<sup>-1</sup> concentrations, as well as two sesame cultivars including ‘Oltan’ (C1) and ‘Dashtestan-2’ (C2) were used as the subplots.

*Field practices*

The field was prepared by using mouldboard and smoothed using disk, and the plots (3 x 3 m) were established during June 2015 and 2016. The seeds were planted in the depth of 1-1.5 cm in the plots, which were 1 m apart including 6 rows (50 cm apart). The field was fertilized according to soil testing using urea (150 kg ha<sup>-1</sup>) and 100 kg of P<sub>2</sub>O<sub>5</sub> per ha in the form of triple superphosphate. The plots were irrigated on a ridge planting and the irrigation time was determined by using Class A evaporation pan (in 70 mm evapotranspiration). Then, the irrigation treatments such as irrigation up to 50% flowering and seed ripening

were performed after foliar application of nutrients. According to the treatments, the irrigation frequency for control, mild, and severe stresses were 8, 7, and 5 times, respectively. The foliar application of nano-Mg ( $2 \text{ g L}^{-1}$ ) and chitosan ( $4.8 \text{ g L}^{-1}$ ) was conducted in 65 BBCH stage and  $6.4 \text{ g L}^{-1}$  chitosan was performed in 75 BBCH stage. Based on the available documents, 75 BBCH is equivalent to seed about 50% of the final size and 65 BBCH equals to 50% of flower open. As for chitosan, chitosan-poly (D-glucosamine) prepared from SIGMA-ALDRICH, and the Khazra nano fertilizer (5% of Mg) were used at  $2 \text{ g L}^{-1}$  concentration for nano-Mg.

#### *Free proline content*

Leaf free proline content was determined by using Bates *et al.* (1973) method, 0.5 g of the fresh leaf was ground with quarts in porcelain pestle and mortar and treated with 10 mL sulfosalicylic acid, and the homogenate was centrifuged at 13000 g for 10 min. Two mL of the filtrated extracts was transferred to tubes with cover, treated with 2 mL of ninhydrin indicator, and 2 mL of glacial acetic acid. The tubes were placed in boiling water at  $100 \text{ }^{\circ}\text{C}$  for 1 h. The tubes were cool down and treated with 4 mL of toluene and shaken with a vortex for 15 to 20 s. The red surface layer and standard samples were simultaneously placed in a spectrophotometer and the absorption of the samples was determined at the wavelength of 520 nanometer. The amount of proline content ( $\mu\text{mol g}^{-1}$  FW) was calculated by using regression equations and the standard curves.

#### *Total carbohydrate content*

In order to estimate the total carbohydrate, 0.1 g sample was placed in a water bath with a Methanol (80%) for 15 min, and centrifuged at 10,000 g for 15 min. The supernatant was used for the quantitative estimation of sugars by the method described by Morris (1948).

#### *Catalase activity (EC 1.11.1.6)*

The CAT activity assay was performed using Chance and Maehly (1955) method. Three mL reaction mixtures containing 2.5 mL 0.05 mM sodium phosphate buffer (pH = 7), and 30  $\mu\text{g}$  protein solution were added to quettes and 30  $\mu\text{L}$   $\text{H}_2\text{O}_2$  (30%) was added to reaction mixture at the time of measurement, and the absorbance was recorded at 240 nanometers, 60 s, and  $25 \text{ }^{\circ}\text{C}$ .

#### *Peroxidase activity (EC 1.11.1)*

The activity of peroxidase enzyme was measured based on Wendel's (1981) method, in which 0.5 g of the plant tissue was homogenized with buffered potassium phosphate (0.2 M at pH= 6.8) by using a cold porcelain pestle, centrifuged at 12000 g for 15 min, and the the solution was used for measuring peroxidase activity. The reaction solution was incubated with 0.25 mL of the raw enzyme, 0.25 mL of guaiacol ( $20 \text{ g L}^{-1}$ ) and 5.2 mL of buffer phosphate (100 M at pH= 6.8) at  $30 \text{ }^{\circ}\text{C}$  for 10 min. The increased absorption was measured 3 min after adding 0.25 mL of  $\text{H}_2\text{O}_2$  with the final concentration of 5 mM using a spectrophotometer at the wavelength of 470 nm. The standard solution (100 mL at pH= 6.8) containing 0.25 mL guayacol 0.5%, 25 mL  $\text{H}_2\text{O}_2$ , 25 mL distilled water, and 2.25 mL buffered phosphate was used.

#### *Ascorbate peroxidase (EC 1.11.1.11)*

The activity of APX was determined according to the following details. 0.2 g fresh plant tissue was homogenized with 5 mL of a buffer solution with 1 mmole ascorbate, 1 mmole EDTA, and 50 mmole potassium phosphate (pH= 7.0) by using a cold porcelain pestle. The extract was then filtrated and centrifuged for 10 min at 10000 g, and the supernatant was used to determine the enzymatic activities. The solution contained 50 mmole potassium phosphate (pH= 0.7), 0.5 mmole ascorbate, 1 mmole  $\text{H}_2\text{O}_2$ , and 1 mmole EDTA, and the total volume of the extracted enzyme was equal to 1 mL. The reaction initiated with  $\text{H}_2\text{O}_2$  and the rate of absorption was measured for 10-30 s at the wavelength of 290 nanometer. The activity of enzyme was determined based on  $\mu\text{mole}$  oxidized ascorbate per minute per mg protein (Asda, 1992).

*Photosynthetic pigments*

In order to measure the content of photosynthetic pigments, the samples were immediately frozen in liquid nitrogen and kept in a freezer at -80 °C before conducting the biochemical analyses. Then, the photosynthetic pigments were measured by using Lichtenthaler (1987) method. Based on this method, 0.25 g of fresh tissue was extracted by using 5 mL 80% acetone. Then, the extract was centrifuged at 11000 rpm for 10 min. In addition, the optical density (O.D.) of the extract was measured at the wavelengths of 646.8 and 663.2 nanometer to estimate chlorophyll a (Chl a) and b (Chl b), respectively, by using a Spectrophotometer. The amount of pigment present in each sample was calculated according to the following equations:

$$\text{Chl a (mg gr}^{-1}\text{ FW)} = 12.7 (\text{O. D of 663}) - 2.69 (\text{O. D of 645}) \times \frac{v}{w \times 1000}$$

$$\text{Chl b (mg gr}^{-1}\text{ FW)} = 22.9 (\text{O. D of 645}) - 4.68 (\text{O. D of 663}) \times \frac{v}{w \times 1000}$$

$$\text{Total Chl (mg gr}^{-1}\text{ FW)} = 20.2 (\text{O. D of 645}) + 8.02 (\text{O. D of 663}) \times \frac{v}{w \times 1000}$$

Where W represents the fresh weight by grams for extracted tissue, V indicates the final size of the extract in 80% acetone, and O.D shows the optical density at a specific wavelength.

*Seed yield and oil content*

Seed yield was determined by collecting plants in a 5-m area at physiological maturity. Further, the amount of seed oil was determined by using the Soxhlet method according to the following details. The seeds were first dried using autoclave and then powdered. Using a cellulose cartridge, the samples were placed in the upper part of the Soxhlet apparatus. The volatilization of the solvent diethyl ether in the bottom of the apparatus resulted in solubilizing the oil, which was isolated, collected and weighed following the evaporation of the solvent. Then, oil percentage was multiplied in the seed yield in order to calculate the oil yield.

*Statistical analyses*

After checking the normality data distribution of data (Kolmogorov-Smirnov and Shapiro-Wilk test) assumption, the studied traits were statistically analysed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2). The differences among means were measured by using least significant difference test (LSD) at 0.05 statistical probability level and the graphs were drawn by MS-Excel.

**Results***Free proline content*

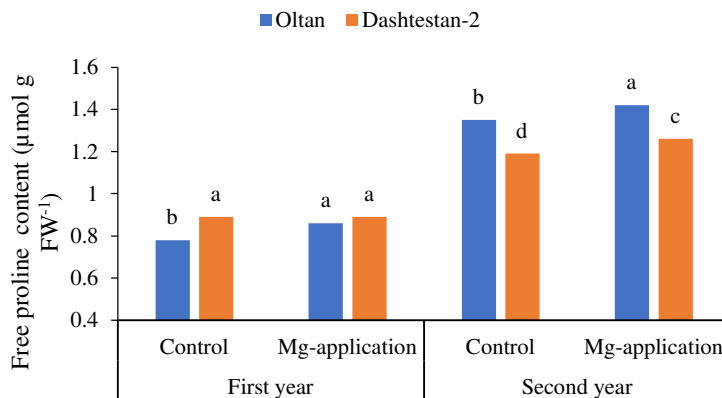
The effect of irrigation (I), Mg nano-chelate (Mg), chitosan (CH), and cultivar (C) were significant on free proline content in both years of the experiment (Table 2). Based on the results, as shown in Table 2, irrigation up to flowering (severe stress=I<sub>3</sub>) caused an increase in free proline content during two years, compared to normal irrigation (I<sub>1</sub>) (13.04 and 62.9%). In addition, the application of Mg nano-chelate and chitosan resulted in increasing the content of free proline significantly during both years (Table 2). Further, 'Dashtestan' cultivar had the highest value of trait in both years. Regarding the interaction Mg × C, the highest free proline content was related to the application of Mg in both cultivars in the first year and the use of Mg in 'Oltan' in the second year (Figure 3).

**Table 2.** Effect of nano-Mg and chitosan on proline content, total sugars, activity of CAT, POD, and APX enzymes of sesame cultivars under water-limited stress

Treatment	Proline content ( $\mu\text{mol g}^{-1}\text{FW}$ )		Total sugars ( $\text{mg g}^{-1}\text{FW}$ )		CAT (U min.mg protein <sup>-1</sup> )		POD (U min.mg protein <sup>-1</sup> )		APX (U min.mg protein <sup>-1</sup> )	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
<b>Irrigation (I)</b>										
I <sub>1</sub>	0.80	0.97	10.47	9.78	2.87	1.71	1.81	1.40	8.31	4.16
I <sub>2</sub>	0.86	1.42	10.62	10.39	4.26	3.53	2.47	2.23	10.74	5.33
I <sub>3</sub>	0.92	1.54	10.97	11.20	4.95	3.91	5.46	4.09	18.05	9.81
St D ( $\pm$ )	0.11	0.17	0.53	0.53	1.49	1.23	0.79	0.75	4.43	3.91
LSD ( $P=0.05$ )	0.86	0.07	0.34	0.28	0.58	0.29	0.43	0.33	4.05	0.95
<b>Nano-Mg</b>										
Control	0.84	1.27	10.62	10.39	4.18	3.20	3.34	2.65	12.61	6.49
Application	0.87	1.34	10.75	10.52	3.87	2.90	3.15	2.50	12.12	6.38
St D ( $\pm$ )	0.12	0.28	0.59	0.80	1.72	1.56	1.81	1.38	6.22	4.82
LSD ( $P=0.05$ )	NS	0.04	NS	NS	0.23	0.15	NS	NS	NS	NS
<b>Chitosan (CH)</b>										
CH1	0.82	1.27	10.39	10.17	4.03	2.98	3.17	2.57	15.13	9.81
CH2	0.91	1.36	10.67	10.44	3.84	2.94	2.58	1.94	8.89	3.86
CH3	0.84	1.29	10.99	10.76	4.21	3.24	3.99	3.21	13.09	5.63
St D ( $\pm$ )	0.12	0.29	0.51	0.74	1.69	1.54	1.71	1.29	5.55	3.95
LSD ( $P=0.05$ )	0.05	0.05	0.23	0.23	0.28	0.19	0.29	0.23	1.67	1.40
<b>Cultivar (C)</b>										
'Oltan' (C1)	0.82	1.23	10.66	10.53	5.05	3.94	3.38	2.71	12.67	6.77
'Dashtestan-2' (C2)	0.89	1.39	10.71	10.38	3.00	2.16	3.11	2.44	12.07	6.09
St D ( $\pm$ )	0.12	0.28	0.61	0.82	1.39	1.29	1.80	1.39	6.23	4.89
LSD ( $P=0.05$ )	0.04	0.04	NS	NS	0.23	0.15	0.24	0.19	NS	NS
<b>Interaction effect</b>										
I $\times$ Mg	NS	**	*	*	**	*	**	**	NS	NS
I $\times$ CH	NS	NS	**	**	**	**	*	**	**	**
I $\times$ C	NS	**	NS	NS	*	**	NS	*	NS	NS
Mg $\times$ CH	NS	NS	NS	NS	*	*	NS	NS	NS	*
Mg $\times$ C	*	*	NS	NS	**	**	NS	*	NS	NS
CH $\times$ C	NS	NS	NS	NS	**	**	NS	NS	NS	NS
I $\times$ Mg $\times$ CH	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
I $\times$ Mg $\times$ C	NS	NS	*	NS	*	**	NS	*	NS	NS
I $\times$ CH $\times$ C	NS	NS	NS	NS	**	**	NS	NS	NS	NS
Mg $\times$ CH $\times$ C	NS	NS	NS	NS	*	**	NS	NS	NS	NS
I $\times$ Mg $\times$ CH $\times$ C	NS	NS	NS	NS	NS	*	NS	*	NS	NS

LSD: least significant difference; NS: non-significant, \* and \*\* significant 5 and 1% probably levels.

I1: normal irrigation; I2: irrigation cut-off in seed ripening (75 BBCH stage); I3: irrigation cut-off in flowering (65 BBCH stage); CH1: non-application of chitosan; CH2: 4.6 g L<sup>-1</sup>; CH3: 6.4 g L<sup>-1</sup>;



**Figure 3.** Free proline content affected by different sesame cultivars and nano-Mg chelate treatments, columns (for each year) with different letters are significantly different at  $P=0.05$ , using LSD test

#### Total sugars

As indicated in Table 2, the effect of I, CH,  $I \times Mg$ , and  $I \times CH$  was significant on the total sugars in both years. The results indicated that the severe stress ( $I_3$ ) and the application of  $6.4 \text{ g L}^{-1}$  chitosan led to an increase in the total sugars in both years (Table 2). In the interaction  $I \times Mg$ , the maximum rate of total sugars was related to non-application of Mg under  $I_3$  by  $11.0$  and  $11.2 \text{ mg g}^{-1} \text{ FW}$  in the first and second years, respectively, while the lowest was related to non-application of Mg under  $I_1$  (normal irrigation) by  $10.2$  and  $9.5 \text{ mg g}^{-1} \text{ FW}$  in the first and second years, respectively (Table 3). Regarding the interaction  $I \times CH$ , the maximum rate of sugars was created by  $CH_3$  ( $6.4 \text{ g L}^{-1}$  chitosan) and  $I_3$  by  $11.7$  and  $11.9 \text{ mg g}^{-1} \text{ FW}$  in the first and second years, respectively, and the minimum was related to  $I_1$  and  $CH_1$  (non-application of chitosan) by  $10.3$  and  $9.6 \text{ mg g}^{-1} \text{ FW}$  in the first and second years, respectively (Table 3).

#### Antioxidant enzyme activities

As shown in Table 2, the effect of I, CH, and  $I \times CH$  was significant on CAT, POD, and APX activities. Based on the results, the limited irrigation ( $I_3$ ) results in increasing CAT, POD, and APX activities and the lowest activity is related to  $I_1$  treatment (Table 2). Regarding the interaction  $I \times Mg$ , the highest CAT and POD activities were related to CAT:  $5.1$  and  $4.1$ , and POD:  $5.5$  and  $4.4 \text{ U min.mg protein}^{-1}$  for the first and second years, respectively. As shown in Table 3, the lowest activities were observed in  $I_1$  in both levels of Mg treatment. However, CAT activity was in the range of  $5.5$  and  $4.2$  in first and second years for  $I_3 \times CH_2$  and  $2.6$  and  $1.5 \text{ U min.mg protein}^{-1}$  in the first and second years for  $I_1 \times CH_2$  (Table 3). A similar result was observed for POD activity, which was in the range of  $6.5$  and  $4.9$  in the first and second years for  $I_3 \times CH_3$  and  $1.4$  and  $1.1 \text{ U min.mg protein}^{-1}$  in the first and second years for  $I_1 \times CH_2$  (Table 3). In addition, the highest APX activity ( $22.79$  and  $16.5 \text{ U min.mg protein}^{-1}$  in the first and second years, respectively) was achieved in the non-application of chitosan under  $I_3$ . As illustrated in Figure 4, the lowest trait was related to the application of  $6.4$  and  $4.8 \text{ g L}^{-1}$  chitosan under  $I_1$  and  $I_2$ , respectively.

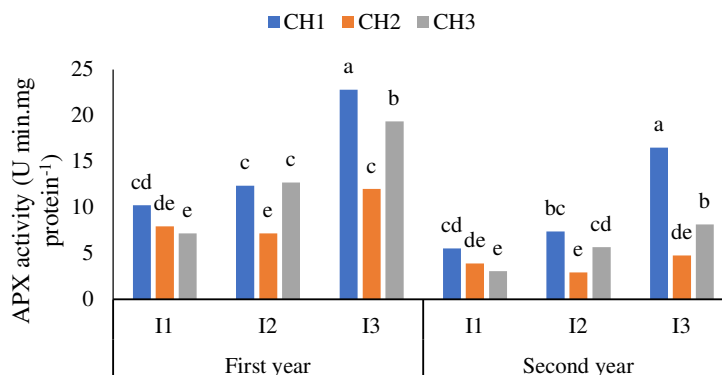


**Table 3.** Total sugars, CAT and POD activities, seed yield, oil percentage and yield affected by irrigation (I) × nano-Mg (Mg) and irrigation × chitosan (CH) treatments during two years

I × Mg		Total sugars (mg g <sup>-1</sup> FW)		CAT (U min.mg protein <sup>-1</sup> )		POD (U min.mg protein <sup>-1</sup> )		Seed yield (kg ha <sup>-1</sup> )		Oil percentage (%)		Oil yield (kg ha <sup>-1</sup> )	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
I1	Control	10.2 c	9.5 d	2.9 d	1.7 d	1.9 c	1.5 d	948.7 b	978.8 b	48.2 a	47.7 b	462.8 b	469.5 b
	Application	10.7 ab	10.0 c	2.7 d	1.6 d	1.7 c	1.2 d	1112.0 a	1293.9 a	48.4 a	52.8 a	545.7 a	687.4 a
I2	Control	10.5 b	10.3 bc	4.4 b	3.6 b	2.5 b	1.2 d	537.9 d	652.2 d	41.3 c	41.8 d	225.3 d	274.5 d
	Application	10.6 b	10.4 b	4.0 c	3.3 c	2.3 b	2.1 c	727.4 c	816.2 c	44.4 b	43.7 c	326.2 c	358.4 c
I3	Control	11.0 a	11.2 a	5.1 a	4.1 a	5.5 a	4.4 a	459.5 e	467.9 f	37.4 d	38.8 e	173.6 e	182.9 f
	Application	10.8 ab	11.1 a	4.7 ab	3.6 b	5.4 a	3.7 b	507.9 d	519.5 e	36.9 d	41.2 d	189.7 e	215.0 e
I × CH													
I1	CH1	10.3 c	9.6 f	3.0 d	1.6 f	1.7 g	1.1 f	836.9 c	973.9 c	44.0 cd	47.9 c	371.2 c	472.0 c
	CH2	10.5 bc	9.8 ef	2.6 d	1.5 f	1.4 g	1.1 f	1019.2 b	1139.7 b	49.2 b	50.3 b	501.2 b	578.4 b
	CH3	10.5 bc	9.8 ef	2.9 d	1.8 f	2.2 ef	1.9 de	1235.1 a	1295.5 a	51.7 a	52.4 a	640.3 a	685.0 a
I2	CH1	10.3 bc	10.1 de	4.4 b	3.6 cd	2.4 e	2.3 d	504.6 f	614.5 f	40.5 e	41.1 fg	206.1 f	253.9 f
	CH2	10.7 b	10.5bcd	3.9 c	3.1 e	1.7 fg	1.6 e	626.1 e	742.4 e	43.1 d	42.9 de	272.0 d	320.2 e
	CH3	10.7 bc	10.4 cd	4.4 bc	3.8 bc	3.1 d	2.7 c	767.2 d	845.7 d	45.1 c	44.2 d	349.1 c	375.3 d
I3	CH1	10.4 bc	10.7 bc	4.0 bc	3.4 d	5.3 b	4.2 b	373.0 g	415.7 i	34.6 g	37.9 h	128.9 g	158.1 h
	CH2	10.6 bc	10.9 b	5.5 a	4.2 a	4.5 c	3.0 c	485.1 f	492.2 h	36.9 f	40.0 g	179.2 f	197.5 g
	CH3	11.7 a	11.9 a	4.2 a	4.0 ab	6.5 a	4.9 a	592.9 e	573.2 g	39.9 c	42.1 ef	236.7 e	241.3 f

Columns (for each year) with different letters are significantly different at  $P=0.05$ , using LSD test.

I1: normal irrigation; I2: irrigation cut-off in seed ripening (75 BBCH stage); I3: irrigation cut-off in flowering (65 BBCH stage); CH1: non-application of chitosan; CH2: 4.6 g L<sup>-1</sup>; CH3: 6.4 g L<sup>-1</sup>;



**Figure 4.** APX activity affected by water-limited and chitosan treatments, columns (for each year) with different letters are significantly different at  $P=0.05$ , using LSD test

*Photosynthetic pigments*

Based on the results, I, CH, and Mg × cultivars (C) affected chlorophyll a, b, and the total contents significantly (Table 4). In addition, as shown in Table 4, the irrigation up to flowering (severe stress) caused a significant decrease in chlorophyll a and the total, while irrigation up to seed ripening (mild stress) could increase chlorophyll b, compared to the control treatment (I<sub>1</sub>). Further, the application of chitosan resulted in increasing chlorophyll a and the total contents. ‘Dashtestan-2’ cultivar had higher chlorophyll a and the total, compared to the amount in ‘Oltan’. However, ‘Oltan’ had the highest chlorophyll b content (Table 4). As illustrated in Fig. 5, the highest chlorophyll a content in interaction Mg × C was related to the application of Mg in both cultivars and non-application of Mg in ‘Dashtestan-2’ and the lowest was related to the non-application of Mg in ‘Oltan’ cultivar during the two years of the experiment. Further, the non-application of Mg in ‘Dashtestan-2’ had the highest content of chlorophyll b 7.76 and 8.36 μg g<sup>-1</sup> FW in the first and second years, respectively). However, as shown in Fig. 6, the lowest was related to the application of Mg in ‘Dashtestan-2’ (6.53 and 7.01 μg g<sup>-1</sup> FW in the first and second years, respectively). In the first and second years, the total

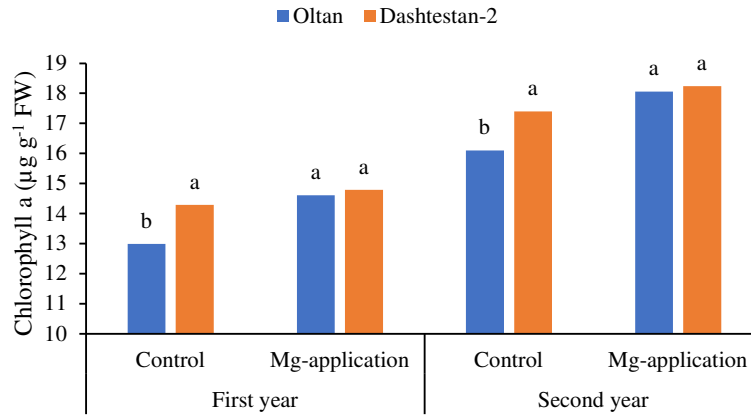
chlorophyll content was in the range of 22.22 and 26.15 for the application of Mg in ‘Oltan’ to 20.1 and 23.82  $\mu\text{g g}^{-1}$  FW for the non-application of Mg in ‘Oltan’, respectively (Figure 5).

**Table 4.** Effect of nano-Mg and chitosan on chlorophyll a, b, and total of sesame cultivars under water-limited stress

Treatment	Chlorophyll a ( $\text{mg g}^{-1}$ FW)		Chlorophyll b ( $\text{mg g}^{-1}$ FW)		Total chlorophyll ( $\text{mg g}^{-1}$ FW)	
	2015	2016	2015	2016	2015	2016
<b>Irrigation (I)</b>						
I <sub>1</sub>	15.31	18.98	8.21	8.48	23.52	27.46
I <sub>2</sub>	15.08	18.27	8.33	8.96	23.42	27.23
I <sub>3</sub>	12.11	15.09	5.22	5.96	17.33	21.05
St D ( $\pm$ )	1.87	2.11	2.28	2.55	2.60	2.72
LSD ( $P=0.05$ )	1.31	1.36	1.07	0.88	0.95	1.80
<b>Nano-Mg</b>						
Control	13.63	16.75	7.44	8.04	21.08	24.79
Application	14.70	18.15	7.07	7.55	21.77	25.71
St D ( $\pm$ )	2.31	2.60	2.68	2.86	3.89	4.03
LSD ( $P=0.05$ )	0.63	0.62	NS	NS	NS	NS
<b>Chitosan (CH)</b>						
CH1	13.63	16.69	7.94	8.03	21.58	27.72
CH2	14.45	17.21	7.15	9.13	21.60	26.34
CH3	14.41	18.44	6.67	6.23	21.09	24.68
St D ( $\pm$ )	2.34	2.60	2.66	2.62	3.92	3.99
LSD ( $P=0.05$ )	0.78	0.76	1.02	1.07	NS	1.14
<b>Cultivar (C)</b>						
‘Oltan’ (C1)	13.79	17.08	7.36	7.90	21.16	24.99
‘Dashtestan-2’ (C2)	14.54	17.82	7.15	7.69	21.69	25.51
St D ( $\pm$ )	2.33	2.67	2.69	2.87	3.89	4.03
LSD ( $P=0.05$ )	0.63	0.62	0.83	NS	0.88	NS
<b>Interaction effect</b>						
I $\times$ Mg	*	**	NS	NS	NS	NS
I $\times$ CH	**	**	NS	NS	NS	NS
I $\times$ C	NS	NS	*	*	*	*
Mg $\times$ CH	NS	NS	NS	NS	*	*
Mg $\times$ C	*	*	*	*	**	**
CH $\times$ C	NS	NS	NS	NS	NS	NS
I $\times$ Mg $\times$ CH	NS	NS	*	*	NS	NS
I $\times$ Mg $\times$ C	NS	NS	NS	NS	NS	NS
I $\times$ CH $\times$ C	NS	NS	NS	NS	NS	NS
Mg $\times$ CH $\times$ C	NS	NS	NS	NS	NS	NS
I $\times$ Mg $\times$ CH $\times$ C	NS	NS	NS	NS	NS	NS

LSD: least significant difference; NS: non-significant, \* and \*\* significant 5 and 1% probably levels.

I1: normal irrigation; I2: irrigation cut-off in seed ripening (75 BBCH stage); I3: irrigation cut-off in flowering (65 BBCH stage); CH1: non-application of chitosan; CH2: 4.6  $\text{g L}^{-1}$ ; CH3: 6.4  $\text{g L}^{-1}$ ;



**Figure 5.** Chlorophyll a content affected by different sesame cultivars and nano-Mg chelate treatments, columns (for each year) with different letters are significantly different at  $P=0.05$ , using LSD test

#### Seed yield

As shown in Table 5, the effect of I, Mg, CH, C,  $I \times Mg$ ,  $I \times CH$  on seed yield was significant. Further, severe stress caused a decrease in 53.0 and 56.5% during the first and second years, respectively, compared to the amount in the normal irrigation. Regarding the comparison of mean effects, the application of Mg and chitosan enhanced seed yield significantly, compared to the amount in the control treatment (Table 5). Furthermore, ‘Dashtestan-2’ cultivar had the highest seed yield, compared to ‘Oltan’ cultivar. In addition, the highest seed yield in the interaction  $I \times Mg$  was achieved by applying Mg in  $I_1$  treatment (1112 and 1293.9 kg ha<sup>-1</sup> for the first and second years, respectively). As indicated in Table 3, the lowest trait was related to the non-application of Mg in  $I_3$  (459.5 and 467.9 kg ha<sup>-1</sup> for the first and second years, respectively). Regarding the interaction  $I \times CH$ , the seed yield ranged from 1235.1 and 1295.5 ( $I_1$  and  $CH_3$  for the first and second years) to 373.0 and 415.7 kg ha<sup>-1</sup> ( $I_3$  and  $CH_1$  for the first and second years) (Table 3).

#### Seed oil content

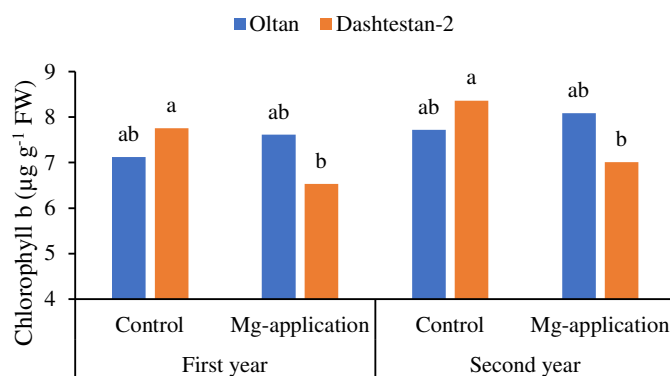
The results indicated that the effect of I, Mg, CH,  $I \times Mg$ , and  $I \times CH$  on the oil percentage and yield was significant in both years (Table 5). In addition, an increase in the level of stress leads to a decrease in the oil percentage and yield under  $I_3$ . Further, adding Mg increased the oil percentage from 42.35 to 43.29 during the first year and 42.82 to 45.92% during the second year). Furthermore, as shown in Table 5, oil yield increased from 287.2 to 353.8 in the first year, and 309.0 to 420.3 kg ha<sup>-1</sup> in the second year). Additionally, an increase occurred in seed oil percentage and yield under chitosan application, compared to the amount in the control treatment (Table 5). As indicated in Table 3, the highest oil percentage (48.4 and 52.8%) and oil yield (545.7 and 687.4 kg ha<sup>-1</sup>) were related to the application of Mg under  $I_1$ . However, the lowest oil percentage (37.4 and 38.8%) and oil yield (173.6 and 182.9 kg ha<sup>-1</sup>) were observed in the non-application of Mg under  $I_3$  (Table 3). Regarding the interaction  $I \times CH$ , the oil percentage ranged 34.6 and 37.9 in the first and second year in  $I_3$  and  $CH_1$  to 51.7 and 52.4% in the first and second years in  $I_1$  and  $CH_3$ . Finally, as shown in Table 3, the highest oil yield was related to  $I_1$  and  $CH_3$  (640.3 and 685.0 kg ha<sup>-1</sup>) while the lowest belonged to  $I_3$  and  $CH_1$  (128.9 and 158.1 kg ha<sup>-1</sup>).

**Table 5.** Effect of nano-Mg and chitosan on seed yield, oil percentage and yield of sesame cultivars under water-limited stress

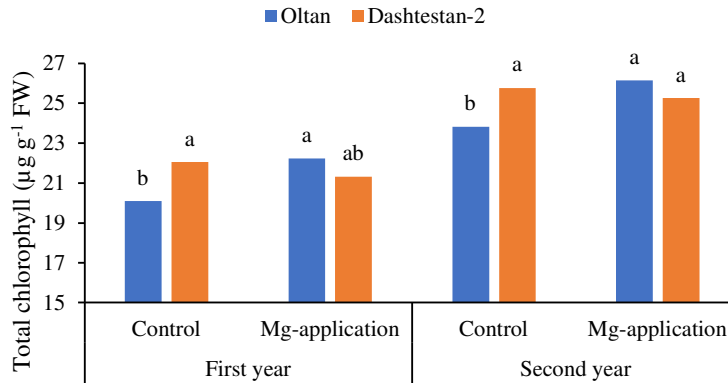
Treatment	Seed yield (kg ha <sup>-1</sup> )		Oil percentage (%)		Oil yield (kg ha <sup>-1</sup> )	
	2015	2016	2015	2016	2015	2016
<b>Irrigation (I)</b>						
I <sub>1</sub>	1030.4	1136.3	48.35	50.25	504.3	578.51
I <sub>2</sub>	632.7	734.2	42.93	42.81	275.8	316.50
I <sub>3</sub>	483.7	493.7	37.18	40.05	181.6	199.00
St D (±)	168.1	147.3	3.68	2.97	94.24	89.19
LSD ( <i>P</i> =0.05)	79.2	17.15	2.39	2.87	35.19	32.68
<b>Nano-Mg</b>						
Control	648.7	699.67	42.35	42.82	287.2	309.0
Application	782.4	876.5	43.29	45.92	353.8	420.3
St D (±)	281.0	292.2	5.92	5.03	165.6	175.8
LSD ( <i>P</i> =0.05)	26.51	14.71	0.83	0.74	14.05	10.44
<b>Chitosan (CH)</b>						
CH1	571.5	668.0	39.71	42.36	235.4	294.6
CH2	710.1	791.4	43.12	44.48	317.5	365.4
CH3	865.1	904.8	45.63	46.27	408.7	433.9
St D (±)	262.3	294.3	5.43	5.08	151.8	179.2
LSD ( <i>P</i> =0.05)	32.47	18.02	1.02	0.91	17.2	12.79
<b>Cultivar (C)</b>						
'Oltan' (C1)	681.7	748.4	42.09	43.82	298.2	341.2
'Dashtestan-2' (C2)	749.4	827.7	43.59	44.92	342.97	388.0
St D (±)	287.0	307.7	5.85	5.27	167.3	187.2
LSD ( <i>P</i> =0.05)	26.51	14.71	0.83	0.74	14.05	10.44
<b>Interaction effect</b>						
I × Mg	**	**	**	**	**	**
I × CH	**	**	*	NS	**	**
I × C	**	**	**	NS	**	**
Mg × CH	NS	**	NS	NS	NS	**
Mg × C	NS	NS	*	NS	NS	NS
CH × C	NS	NS	NS	NS	NS	NS
I × Mg × CH	NS	NS	NS	NS	NS	*
I × Mg × C	*	NS	NS	NS	*	NS
I × CH × C	NS	NS	NS	NS	NS	NS
Mg × CH × C	NS	NS	NS	NS	NS	NS
I × Mg × CH × C	NS	NS	NS	NS	NS	NS

LSD: least significant difference; NS: non-significant, \* and \*\* significant 5 and 1% probably levels.

I1: normal irrigation; I2: irrigation cut-off in seed ripening (75 BBCH stage); I3: irrigation cut-off in flowering (65 BBCH stage); CH1: non-application of chitosan; CH2: 4.6 g L<sup>-1</sup>; CH3: 6.4 g L<sup>-1</sup>;



**Figure 6.** Chlorophyll b content affected by different sesame cultivars and nano-Mg chelate treatments, columns (for each year) with different letters are significantly different at *P*= 0.05, using LSD test



**Figure 7.** Total chlorophyll content affected by different sesame cultivars and nano-Mg chelate treatments, columns (for each year) with different letters are significantly different at  $P=0.05$ , using LSD test

## Discussion

In the present study, a set of properties related to sesame medicinal plant including seed yield, oil content, photosynthetic pigments, antioxidant enzyme activities were determined by limited irrigation regimes and application of chitosan and magnesium-nano fertilizers. The results of ANOVA indicated that the effect of limited irrigation regimes and the application of chitosan and Mg nano-chelate on physiological traits such as free proline content, total sugars, CAT, POD, and APX activities, as well as on photosynthetic pigments such as chlorophyll a, b, and total, seed yield, and oil percentage and yield were significant. In addition, a significant difference was reported between the two cultivars. The superior cultivar in both years was related to 'Dashtestan-2', which resulted in creating higher seed yield, oil percentage, and yield, compared to 'Oltan' cultivar under stress condition. Further, 'Oltan' cultivar had a more positive response to the application of magnesium fertilizer than photosensitive pigments. Furthermore, 'Oltan' had a more positive reaction to the application of magnesium fertilizer, compared to the 'Dashtestan-2' cultivar in term of photosynthetic pigments.

In addition, increased levels of limited irrigation enhanced the rate of free proline significantly in both years. On the other hand, free proline content in the second year was higher than that of the first year. The increased production of free proline under water-limited stress is considered as the most sensitive osmotic responses of plants (Omidi *et al.*, 2018). Further, water-limited stress increased the production of free proline content and the total soluble carbohydrates, and decreased plant growth, water-related properties, and carotenoids, and chlorophyll concentrations. Furthermore, proline improved plant water properties by adjusting osmoregulation because the cultivars ('Dashtestan-2') with higher proline rate had higher growth and yield (Omidi *et al.*, 2018).

Likewise, the results of the present study indicated that the rate of proline, sugars, and CAT, POD, and APX activities increased under irrigation up to flowering ( $I_3$ ), compared to the amount in the control treatment ( $I_1$ ). The results are consistent with those of other studies which indicated that the production of antioxidant enzymes and phenolic compounds increases under water stress condition, which is a mechanism used by plants to avoid the unfavourable effects of water stress (Varela *et al.*, 2016). Additionally, the rate of free proline and protein contents, as well as the activity of antioxidants including superoxide dismutase and APX, increased in drought-stressed plants.

The results in the present study indicated that no significant difference was observed between control ( $I_1$ ) and mild stress ( $I_2$ ) conditions in the rate of chlorophyll a, b, and the total content. However, the values of

the traits decreased under severe stress (I<sub>3</sub>). In addition, the reduction in chlorophyll a, b, and the total contents was reported in sunflower cultivars under severe water stress conditions.

However, a reduction occurred in seed yield, oil percentage, and yield of sesame seed under water-limited stress, while the use of Mg nano-chelate resulted in increasing the value of traits compared to the non-application of treatment in both years. The decrease in oil content due to water stress can be related to the disturbance in the metabolic processes of seeds and damage to the transfer of assimilates to seeds. The results are in line with those in some previous studies (Morteza *et al.*, 2013). In general, Mg plays an important role in both photosynthesis and protein-synthesis in leaves. Latent Mg deficiencies are common, and Mg addition indicated an improved yield (Xiao *et al.*, 2018). Mg deficiency in the plant reduced the amount of chlorophyll and resulted in decreasing the growth of the plant. In another study, the use of Mg increased seed yield of sesame significantly. In the flowering stages, an increase occurs in the plant need for the nutrient. Thus, the availability of sufficient nutrients results in increasing seed yield and oil content. Some believe that the oil content is controlled by genetic factors, but the availability of essential elements in the critical stage of plant life can affect the rate of photosynthesis and production of plant metabolites, and ultimately help to accumulate oil and enhanced growth (Nikolova and Popp, 2013; Kaluzewicz *et al.*, 2017).

In addition, the results indicated that applying 6.4 g L<sup>-1</sup> increases seed yield and oil content in chitosan treatment in both years. Researchers reported that the application of chitosan on soybean increases the rate of yield significantly, compared to the non-application case. The use of chitosan foliar plays a role in reducing the diameter of the stoma to prevent from depleting cell water, which leads to an increase in the biomass and final yield. Further, chitosan contributes to an increase in photosynthesis by enhancing the chlorophyll content, which can be regarded as one of the reasons for increasing seed yield in the present study (Zeng *et al.*, 2012). Furthermore, chitosan may be facilitated by controlling the wastewater pathways and increasing root area levels in order to increase cell bioactivity and the oil yield.

Finally, the superiority of 'Dashtestan-2' is more than genetic variation, compared to 'Oltan' cultivar. Based on the results, 'Oltan' cultivar has a high reactivity to the application of Mg nano-chelate and chitosan. The seed yield and oil content during the second year were higher than the amounts in the first year, due to the favourable climate conditions in terms of temperature and precipitation in the second year.

## Conclusions

The results of the present study indicate that the yield and physiological properties including proline content, total sugars, activity of antioxidant enzymes such as CAT, POD, and APX, photosynthetic pigments, seed yield, and oil content of different cultivars in sesame, as a medical plant, may be differently affected by foliar application of nano-Mg and chitosan and water-limited stress. Further, the plant uses some avoiding and tolerating mechanisms such as an increase in proline, sugars, activity of CAT, POD, and APX enzymes in order to survive under severe water stress. Further, such tolerance is the result of some physiological and biochemical activities in the plant among which the increased production of proline, sugars, activity of CAT, POD, and APX enzymes are highlighted as the most important ones, which are related to both medicinal and defensive values. Additionally, 'Dashtestan-2' cultivar had more tolerant cultivar under water stress. It is worth noting that the foliar application of nano-Mg and chitosan fertilizers leads to the increased production of the total chlorophyll, seed yield, and oil content. Furthermore, the co-application of nano-Mg and CH2 (4.6 g L<sup>-1</sup> chitosan) increased the production of proline and sugars significantly while decreased the activity of CAT, POD, and APX enzymes, which may be regarded as one of the physiological reasons of sesame cultivars for the water-limited stress condition. Thus, nano-Mg and chitosan foliar application are recommended in sesame fields, especially under water-limited supply condition. Future research can be conducted on evaluating the foliar application of nano-Mg and chitosan on oil quality of sesame under water-limited stress, which is more efficient in the production of oilseed crops.

### Authors' Contributions

All authors read and approved the final manuscript.

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### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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