



## Physiological response of sesame (*Sesamum indicum* L.) to application of chitosan and magnesium-nano fertilizers under irrigation cut-off in a sustainable agriculture system

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### Abstract

In order to investigate chitosan and magnesium-nano fertilizers' impact on photosynthetic pigments, protein, proline, and soluble sugar contents of sesame under irrigation cut-off treatment, a split-factorial experiment was conducted based on randomized blocks with three replications. Irrigation cut-off based on BBCH scale as the main factor (normal irrigation and irrigation cut-off in 6 and 75 BBCH stages). Secondary factors as subplot included Oltan and Dashtestan-2 sesame cultivars, and foliar application of Mg-nano fertilizer (application and non-application) and chitosan (control, foliar application of 4.8 g.l<sup>-1</sup> in 65 BBCH and 6.4 g.l<sup>-1</sup> in 75 BBCH stages). Based on the results, severe drought stress (irrigation up to 65 BBCH) resulted in reducing the mean of chlorophyll a, b, and total compared to the control treatment. Chitosan foliar application yielded more desirable results compared to those of Mg-nano fertilizer and caused an increase in the mean traits of chlorophyll a, b, total, carotenoid, protein, proline, and soluble sugar. In addition, irrigation up to 65 BBCH (severe drought stress) and non-application of nanofertilizer led to a decrease in chlorophyll content and physiological damage. Based on the findings, chitosan biopolymer, as a natural substance, as well as co-application of these two can be an appropriate action in order to decrease the plant damage under drought stress regarding the role of Mg in chlorophyll structure and a large number of the plant vital enzymes.

**Keywords:** BBCH scale; drought stress; nutrient function; osmotic adjustment; photosynthetic pigments

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### Introduction

Sesame (*Sesamum indicum* L.) is an herbaceous annual plant belonging to the Pedaliaceae family and is one of the oldest oilseed herbs which is adapted to the arid and semi-arid

areas and known as the queen of the oilseed plants (Roul et al., 2017). Regarding sesame farming, drought stress is one of the factors which reduce plant growth and prevent photosynthesis (Efeoglu et al., 2009). High chlorophyll index indicates plant health, though drought stress reduces this index and ultimately reduces the efficiency of photosynthesis (Lotfi et al., 2015).

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Research has shown that plant dehydration (i.e. under drought stress) generally leads to the destruction and breakdown of chloroplasts and reduction in chlorophyll content and the activity of enzymes in the Calvin cycle during the photosynthesis process (Monakhova and Chernyader, 2002). Similarly, Mafakheri et al. (2010) reported the reduced leaf chlorophyll content due to drought stress. Interference with the cellular metabolism of the plant is associated with the decomposition of proteins (Sakata et al., 2014). Jabasingh and Saravana Babu (2014) hold that protein content in corn decreases with increasing dehydration stress and this reduction may be regarded as an adaptation strategy b plants against dehydration. Farouk and Amany (2012) believe that plants go along with the drought in dry circumstance toward different ways such as closing the stomata, osmotic regulation, and the accumulation of compatible soluble materials with dryness. Proline as an osmotic regulator, plays a key role in maintaining water balance, preserving protein stability, stabilizing membranes and protein synthesis system, providing storing carbon and nitrogen for growth after stress relief, reducing the risks of reactive oxygen species (ROS) production (Verbruggen and Hermans, 2008). Soluble sugars act as osmotic regulators, cell membrane stabilizers, and cellular turgor controller (Slama et al., 2007). In addition, plants with high soluble sugar accumulation can respond efficiently to drought stress through osmotic adjustment (Slama et al., 2007). In another study conducted by Valentovic et al. (2006) proline and soluble sugar contents increased in facing with drought stress.

Using nanofertilizers to control the release of nutritional elements can be an effective step towards sustainable and eco-friendly agriculture. Magnesium (Mg) is essential for the

chlorophyll synthesis and contributes up to 10% of the total Mg in the chlorophyll structure (Wilkinsan et al., 1991).  $Mg^{2+}$  is involved in several vital processes of plants including the formation of ATP in chloroplasts,  $CO_2$  stabilization, protein synthesis, chlorophyll formation, development of phloem, and optical oxidation in leaves (Cakmak and Yazici, 2010). Mg activates a large number of enzymes such as vas ATP-as enzymes, RuBP, RNA polymerases, and protein kinase (Cakmak and Yazici, 2010). Generally, the first reaction of plants to Mg scarcity affect chlorophyll content. Kumar et al. (2006) emphasized that chlorophyll and carotenoids in rice plant decrease due to Mg deficiency while an increase in Mg in wheat plant induces chlorophyll accumulation (EL-Metwally et al., 2010). Mg is a critical element which is required to maintain a high pH level in chloroplasts and cytoplasm. The effect of pH on the protein structure depends on pH regulation. In this matter, cations such as Mg can play as pH regulators. According to Wang et al. (2004), the concentration of protein and soluble sugars in plant leaves decreases in the Mg insufficiency condition. Further,  $Mg^{2+}$  deficiency reduces the proline content which has been confirmed by Lesko et al. (2002).

Chitosan is a linear biopolymer with n-acetyl-glucosamine monomers with linear bonds (4 to  $\beta$  1). Chitosan seems to act as a cellular signal to make gene expression responding to stress (Ebrahimzadeh and Bahramian, 2009) and may increase the plant resistance to environmental stresses. The mechanism of chitosan activity is not well-known in reducing the harmful effects of drought and a few studies have been conducted in this field. After chitosan foliar application, it seems that a number of changes occur in the cell membrane, chromatin, DNA, ROS concentration, callus, PR (Pathogenesis-Related), gene/proteins,

Table 1  
 Physical and chemical characteristics of the used soil

Depth cm	pH	EC dS.m <sup>-1</sup>	OC %	TNV %	N %	P ppm	K ppm	clay %	silt %	sand %	Texture
0-30	7.68	5.38	0.55	20.58	0.05	12	284.2	19	46	35	loam

Table 2  
Experimental treatments details

Treatments	Levels	BBCB stage
Drought stress (main factor)	Normal irrigation (as control)	-
	Sever stress (irrigation up to flowering)	65
	Moderate stress (irrigation up to seed ripening)	75
Magnesium-nanofertilizer (first subplot)	Control	-
	Foliar application (2 g/Lit)	60
Chitosan (second subplot)	Control (D1)	-
	Foliar application (4.8 g/Lit) (D2)	65
	Foliar application (6.4 g/Lit) (D3)	75
Cultivar (third subplot)	Oltan	-
	Dashtestan-2	

phytoalexins, oxidative burst, and the activity of the MAP kinase pathway (Hadwiger, 2013). In some other studies, chitosan foliar application caused an increase in photosynthetic pigmentation (Pongprayoon et al., 2013), soluble sugars (Saif Eldeen et al., 2014), soluble protein (Sultana et al., 2017) and proline content (Mahdavi et al., 2011).

In addition, the evaluation of the role of nanofertilizers as an approach to reduce the negative effects of stress in plants is important. The present study aimed to investigate the relationship between drought stress and Mg application in the form of nanofertilizer and bio-polymer of chitosan on the photosynthetic, soluble protein, and osmotic regulator of sesame plants which were used as the second crop after harvesting wheat to provide a suitable solution to cope with drought stress and a step toward sustainable agricultural practices.

## Methods and Materials

In order to evaluate the application of chitosan and Mg-nano fertilizers on some physiological traits of two sesame cultivars (Oltan and Dashtestan-2) in the second cultivation a factorial split plot experiment was conducted based on a completely randomized block design with three replications in the research field in Varamin city, South of Tehran, Iran (35° 30' latitudes and 51° 73' longitudes) during 2015-16. Based on the Coupon classification, the weather of the area is identified as BWK. According to the soil test results (Table 1), the farmland has a lumen texture with pH of 7.68. The experimental

treatments are presented in Table 2. The plots were irrigated on a ridge planting and the irrigation time was determined by using Class A evaporation pan (in 70 mm evapotranspiration). After examining physiologically and removing the marginal effects of each plot (3 × 3 m), ten plants were randomly selected. The young leaves of the plants were used to assess the traits change including chlorophyll a, b, total (Arnon, 1949), and carotenoids (Arnon, 1949), proline (Bates et al., 1973), soluble protein (Bradford, 1976), and soluble sugars (Irrigoyen et al., 1992). Finally, the data was collected and analyzed by SAS (9.1) software. Further, comparing the means was done by Duncan multiple test at probability level ( $P \leq 0.05$ ).

## Results

### Photosynthetic pigments

As indicated in Table 3, the effects of drought stress, Mg-nano fertilizer, cultivar, and chitosan × drought stress was significant on the chlorophyll a content. Results indicated that the effect of drought stress and cultivar × drought stress were significant on chlorophyll b and total contents. Also, analysis of variance showed the quadruple interaction between drought stress × Mg-nano × chitosan × cultivar significantly affected total chlorophyll content (Table 3). The highest chlorophyll a content was observed for non-application of chitosan fertilizer under irrigation up to 75 BBCH (mild stress) and foliar application of 4.6 g.L<sup>-1</sup> chitosan fertilizer under irrigation up to 65 BBCH (severe stress), 16.33 and 16.2 mg.g<sup>-1</sup> FW, respectively. The lowest mean of

Table 3

Analysis of variance for the effect of spraying chitosan and manganese-nanofertilizers on some physiological traits of two sesame cultivars (*Sesamum indicum* L.) under drought stress

S.O.V	Mean square (MS)							
	df	Chl a	Chl b	Total Chl	Carotenoids	Proline content	Soluble sugars	Protein content
Block	2	5.02 <sup>ns</sup>	13.98 <sup>ns</sup>	35.3 <sup>ns</sup>	0.81 <sup>ns</sup>	0.005 <sup>ns</sup>	0.296 <sup>ns</sup>	0.66 <sup>ns</sup>
Drought (D)	2	114.7 <sup>**</sup>	111 <sup>**</sup>	451.7 <sup>**</sup>	0.37 <sup>ns</sup>	0.124 <sup>*</sup>	2.35 <sup>*</sup>	24.6 <sup>*</sup>
Block × D	4	4.04	2.68	2.13	2.38	0.017	0.28	1.84
Mg-nano (N)	1	30.56 <sup>*</sup>	3.71 <sup>ns</sup>	12.98 <sup>ns</sup>	8.03 <sup>ns</sup>	0.034 <sup>ns</sup>	0.52 <sup>ns</sup>	10.66 <sup>*</sup>
N × D	2	7.1 <sup>ns</sup>	4.52 <sup>ns</sup>	1.38 <sup>ns</sup>	7.99 <sup>ns</sup>	0.001 <sup>ns</sup>	1.11 <sup>*</sup>	0.29 <sup>ns</sup>
Chitosan (C)	2	7.64 <sup>ns</sup>	14.84 <sup>ns</sup>	2.97 <sup>ns</sup>	8.38 <sup>*</sup>	0.074 <sup>*</sup>	3.19 <sup>**</sup>	150.3 <sup>**</sup>
C × D	4	11.68 <sup>*</sup>	1.98 <sup>ns</sup>	8.44 <sup>ns</sup>	1.74 <sup>ns</sup>	0.005 <sup>ns</sup>	1.36 <sup>**</sup>	29.37 <sup>**</sup>
C × N	2	0.72 <sup>ns</sup>	8.71 <sup>ns</sup>	14.12 <sup>ns</sup>	3.43 <sup>ns</sup>	0.001 <sup>ns</sup>	0.13 <sup>ns</sup>	2.46 <sup>ns</sup>
C × N × D	4	1.93 <sup>ns</sup>	11.2 <sup>ns</sup>	6.78 <sup>ns</sup>	2.4 <sup>ns</sup>	0.007 <sup>ns</sup>	0.34 <sup>ns</sup>	1.16 <sup>ns</sup>
Cultivar (Cul)	1	14.99 <sup>*</sup>	1.29 <sup>ns</sup>	7.49 <sup>ns</sup>	4.67 <sup>ns</sup>	0.12 <sup>**</sup>	0.09 <sup>ns</sup>	7.32 <sup>**</sup>
Cul × D	2	0.97 <sup>ns</sup>	17.15 <sup>*</sup>	26.06 <sup>*</sup>	2.2 <sup>ns</sup>	0.005 <sup>ns</sup>	0.06 <sup>ns</sup>	0.07 <sup>ns</sup>
Cul × N	1	8.59 <sup>ns</sup>	19.94 <sup>ns</sup>	54.7 <sup>**</sup>	0.65 <sup>ns</sup>	0.044 <sup>ns</sup>	0.22 <sup>ns</sup>	0.72 <sup>ns</sup>
Cul × C	2	0.55 <sup>ns</sup>	3.14 <sup>ns</sup>	6.05 <sup>ns</sup>	0.06 <sup>ns</sup>	0.006 <sup>ns</sup>	0.002 <sup>ns</sup>	0.26 <sup>ns</sup>
Cul × D × N	2	0.54 <sup>ns</sup>	0.02 <sup>ns</sup>	0.75 <sup>ns</sup>	0.25 <sup>ns</sup>	0.011 <sup>ns</sup>	0.62 <sup>ns</sup>	1.62 <sup>ns</sup>
Cul × D × C	4	1.37 <sup>ns</sup>	2.22 <sup>ns</sup>	5.9 <sup>ns</sup>	0.57 <sup>ns</sup>	0.004 <sup>ns</sup>	0.007 <sup>ns</sup>	0.89 <sup>ns</sup>
Cul × N × C	2	2.62 <sup>ns</sup>	2.06 <sup>ns</sup>	6.08 <sup>ns</sup>	0.61 <sup>ns</sup>	0.008 <sup>ns</sup>	0.40 <sup>ns</sup>	1.63 <sup>ns</sup>
Cul × D × N × C	4	1.34 <sup>ns</sup>	3.29 <sup>ns</sup>	23.42 <sup>*</sup>	0.37 <sup>ns</sup>	0.11 <sup>*</sup>	0.13 <sup>ns</sup>	0.44 <sup>ns</sup>
Error	66	2.4	5.11	5.86	2.11	0.01	0.30	0.77
CV (%)	-	12.93	26.38	9.78	21.77	13.5	4.47	13.06

ns: non-significant; \*and \*\* significant at 0.05 and 0.01 probability level, respectively; Chlorophyll a: Chl a; Chlorophyll b: Chl b; Total chlorophyll: total Chl; Coefficient variation: CV

this trait (11.12 mg.g<sup>-1</sup> FW) was achieved in the foliar application of 6.4 g.L<sup>-1</sup> chitosan fertilizer under normal irrigation (Fig. I). Results illustrated that both cultivars (Oltan and Dashtestan-2) showed the highest means of chlorophyll b content under normal irrigation and irrigation up to 75 BBCH while under severe drought stress (irrigation up to 65 BBCH) the lowest mean of the trait was recorded (Fig. II).

In the quadruple interaction, the highest total chlorophyll was obtained in the co-application of Mg-nano and 4.6 g.L<sup>-1</sup> chitosan or non-application of this treatment in Dashtestan-2 cultivar (26.08 and 26.49 mg.g<sup>-1</sup> FW, respectively). The application of 4.6 g.L<sup>-1</sup> chitosan and non-application of Mg-nano fertilizer in Oltan cultivar under severe drought stress irrigation up to 65 BBCH showed the lowest means of total chlorophyll content (15.6 mg.g<sup>-1</sup> FW) (Table 4).

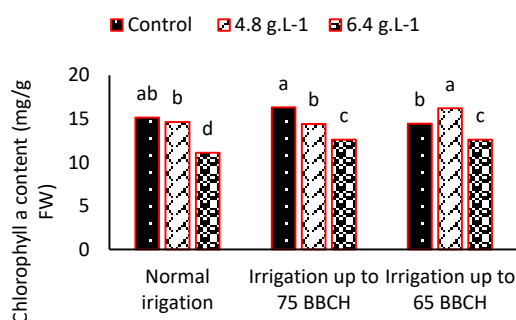


Fig.I. Interaction of drought stress × chitosan treatments on chlorophyll a content of sesame (the same letters show non-significant differences based on Duncan multiple test at P<0.05.)

### Proline content

As shown in Table 3, the effects of drought stress, chitosan, cultivar, and drought stress × Mg-nano foliar application × chitosan × cultivar were

Table 4  
Mean comparison effect of drought stress, Mg-nano and chitosan fertilizers on total chlorophyll and proline contents of sesame cultivars (*Sesamum indicum* L.)

Drought stress	Mg-nano fertilizer	Chitosan	Cultivar	Total chlorophyll (mg.g <sup>-1</sup> FW)	Proline content (μmol.g <sup>-1</sup> FW)
D1	N1	C1	Oltan	20.22±1.44 d-i	0.68±0.07 ef
			Dashtestan2	24.55±3.36 abc	0.83±0.06 b-f
		C2	Oltan	23.29±2.18 a-e	0.77±0.08 c-f
			Dashtestan2	24.83±3.13 ab	0.92±0.07 a-d
		C3	Oltan	20.21±3.43 d-i	0.67±0.13 f
			Dashtestan2	25.04±3.13 ab	0.85±0.06 b-f
	N2	C1	Oltan	23.57±1.88 a-d	0.77±0.17 c-f
			Dashtestan2	26.08±2.61 a	0.75±0.12 c-f
		C2	Oltan	23.97±1.75 a-d	0.88±0.09 a-d
			Dashtestan2	26.49±3.8 a	0.89±0.09 a-d
		C3	Oltan	22.48±0.46 a-f	0.81±0.12 b-f
			Dashtestan2	21.59±1.76 b-g	0.81±0.07 b-f
D2	N1	C1	Oltan	23.96±2.09 a-d	0.72±0.08 def
			Dashtestan2	24.81±3.79 ab	0.88±0.18 a-e
		C2	Oltan	21.83±2.63 b-g	0.88±0.09 a-d
			Dashtestan2	21.69±3.36 b-g	0.88±0.1 a-e
		C3	Oltan	22.66±2.69 a-f	0.76±0.14 c-f
			Dashtestan2	24.81±1.45 ab	0.92±0.13 a-d
	N2	C1	Oltan	25.52±2.09 ab	0.85±0.07 a-f
			Dashtestan2	20.61±2.86 c-h	0.9±0.28 a-d
		C2	Oltan	24.54±1.59 abc	0.87±0.17 a-e
			Dashtestan2	23.09±2.57 a-e	0.9±0.05 a-d
		C3	Oltan	23.97±2.41 a-d	0.83±0.07 b-f
			Dashtestan2	23.57±1.64 a-d	0.94±0.03 abc
D3	N1	C1	Oltan	16.58±1.25 hij	0.81±0.07 b-f
			Dashtestan2	16.39±1.25 ij	0.93±0.05 abc
		C2	Oltan	15.6±2.83 j	0.94±0.11 abc
			Dashtestan2	17.09±2.99 hij	0.94±0.14 abc
		C3	Oltan	16.61±2.37 hij	0.87±0.06 a-e
			Dashtestan2	19.31±3.38 e-j	0.94±0.08 abc
	N2	C1	Oltan	20.49±2.16 c-h	0.89±0.14 a-d
			Dashtestan2	16.26±1.69 ij	0.89±0.08 a-d
		C2	Oltan	18.93±1.88 f-j	0.99±0.06 ab
			Dashtestan2	17.89±2.71 g-j	1.05±0.2 a
		C3	Oltan	16.56±1.85 hij	0.88±0.13 a-d
			Dashtestan2	16.36±0.4 ij	0.89±0.12 a-d

The same letters show non-significant differences based on Duncan multiple test at P≤0.05.

D1: Normal irrigation (non-stress); D2: Irrigation up to 75 BBCH (mild stress); D3: Irrigation up to 65 BBCH (severe stress); N1: control (non-application); N2: foliar application Mg-nano fertilizer; C1: control (non-application); C2: 4.8 g.l<sup>-1</sup> chitosan foliar application; C3: 6.4 g.l<sup>-1</sup> chitosan foliar application

significant on proline content. In the quadruple interaction, the highest level of proline (1.05 μmol.g<sup>-1</sup> FW) was observed in co-application of Mg-nano fertilizer and 4.6 g.l<sup>-1</sup> chitosan in Dashtestan-2 cultivar under severe drought stress (irrigation up to 65 BBCH) which showed 35.2% increase compared to the control treatment. The lowest mean of this trait (0.67 μmol.g<sup>-1</sup> FW) was achieved in the non-application of nanofertilizer with foliar application of 6.4 g.l<sup>-1</sup> chitosan in Oltan

cultivar under normal irrigation (free-stress condition) (Table 4).

### Soluble sugars content

Results showed that the effect of drought stress, chitosan, Mg-nano fertilizer × drought stress, and chitosan × drought stress were significant on soluble sugars content (Table 3).

Table 5  
 Correlation coefficients of the measured traits in sesame genotypes under drought stress, Mg-nano, and chitosan treatments

	Chl a	Chl b	Total Chl	Carotenoids	Proline content	Soluble sugars	Protein content
Chl a	1						
Chl b	0.18 ns	1					
Total Chl	0.73**	0.80**	1				
Carotenoids	0.54**	-0.62**	-0.09ns	1			
Proline content	-0.03 ns	-0.23*	0.18 ns	0.13 ns	1		
Soluble sugar	-0.08 ns	-0.22*	-0.20*	0.10 ns	0.16 ns	1	
Protein content	0.13	0.31**	0.30**	-0.16 ns	-0.06	-0.38**	1

ns: non-significant; \*and \*\* significant at 0.05 and 0.01 probability level, respectively

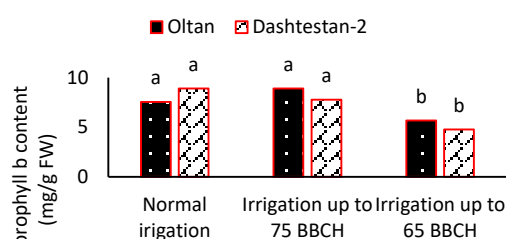


Fig. II. Interaction of drought stress × sesame cultivars (Oltan and Dashtestan-2) on chlorophyll b content (the same letters show non-significant differences based on Duncan multiple test at probability at  $P \leq 0.05$ .)

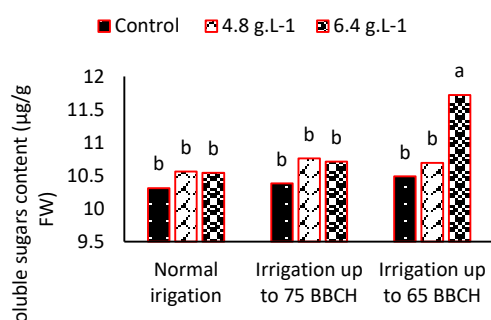


Fig. III. Interaction of drought stress × chitosan treatments on the total sugar content of sesame (the same letters

Under interaction of drought stress and chitosan, the highest mean of this trait was achieved in the irrigation up to 65 BBCH (50% flowering) in 6.4 g.l<sup>-1</sup> application of chitosan (11.72 µg.g<sup>-1</sup> FW). Also, the lowest content was observed in the other treatment (Fig. III). Under interaction of drought stress and Mg-nano fertilizer, the highest mean the soluble sugar contents was achieved at high level of drought stress (irrigation up to 65 BBCH) under non-application of nanofertilizer (11.05 µg.g<sup>-1</sup> FW). Also, the lowest content was observed in non-application of nanofertilizer under normal irrigation treatment (10.21 µg.g<sup>-1</sup> FW) (Fig. IV).

### Soluble protein

Findings indicated that the effect of drought stress, Mg-nano fertilizer, chitosan, cultivar, and drought stress × chitosan was significant on the leaf soluble protein content (Table 3). Under the interaction of drought stress × chitosan, the highest soluble protein content (11.3%) was observed in normal irrigation (free-stress condition) under non-application of chitosan, which showed 18.3% increase compared to the control treatment. The lowest content (5.1%) was achieved in 6.4 g.l<sup>-1</sup> chitosan application under severe drought stress (irrigation up to 65 BBCH) (Fig. V).

### Correlation between traits

As shown in Table 5, total chlorophyll content had a significantly positive correlation with chlorophyll a and b, and protein content, while it showed a negative correlation with soluble sugars. A significantly negative correlation was found between soluble sugars and protein content.

### Discussion

Drought stress in plant tissues leads to stomatal closure and decreased photosynthesis. In this study, chlorophyll a, b, the total chlorophyll, and carotenoids decreased significantly due to drought stress. Molecular degradation of chlorophyll seems to be caused by Phytolus chain separation from the porphyrin ring due to free oxygen radicals or chlorophyllase enzyme (Parvaiz

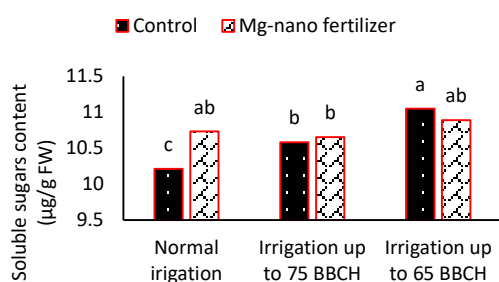


Fig. IV. Interaction of drought stress × Mg-nano treatments on the total sugar content of sesame (the same letters show non-significant differences based on Duncan multiple test at P≤0.05.)

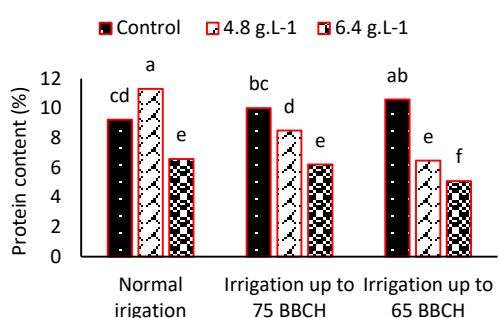


Fig. V. Interaction of drought stress × chitosan treatments on the protein content of sesame (the same letters show non-significant differences based on Duncan multiple test at P≤0.05.)

and Satyawati, 2008). The reduction of photosynthetic pigments in barley plants (Mowludi et al, 2014) and common bean (Sharifa and Muriefah, 2013) has been reported under drought stress conditions. Increasing chlorophyll content of plants by Mg-nano fertilizer treatment may be due to the important role of Mg in the plant life cycle, especially in the chlorophyll production. Reduced levels of chlorophyll and photosynthesis due to the lack of Mg consumption were observed in the study of Yang et al. (2012). In addition, in another study by Taha (2016), Mg foliar application triggered photosynthetic pigments in the squash plant. It seems that the chitosan elicitor activated gene expression and consequently improved the production of chlorophyll in the biosynthesis pathway. Furthermore, researchers have reported that the use of chitosan reduces the effect of drought stress on chlorophyll and increases the photosynthetic pigments (Gornik et al., 2008). The increase is justifiable because of the presence of

nitrogen in chitosan and the structural role of this element in chlorophyll Tetrapyrrole rings. In another study, Limpanavech et al. (2008) found that chitosan plays a role in increasing the content of chlorophyll and photosynthesis. Furthermore, they showed that chitosan can affect the expression of the chloroplast gene so that the variations in the size and development of chloroplasts may be regarded as a stimulating factor in plant growth.

In the present study and other studies, an increase in proline concentration in plant organs was evident with the escalation of water deficit or drought stress. The increase is mostly associated with osmotic regulation of the plant. Increasing proline accumulation during stress conditions takes place due to the stimulation of synthesis through glutamate acid, and the prevention of oxidation in protein synthesis procedure during water shortage (Lutts et al., 1996). Due to dehydration stress, transcription of mRNA is induced by proline-5 carboxylic acid synthase (P5cs) -proline-5 carboxylate reductase (P5CR) enzymes (Liang et al., 2013). Proline accumulation is related to an increase in the activity of the enzymes involved in proline synthesis through glutamate pathway including  $\gamma$ -glutamine kinase, glutamine phosphate reductase, and  $\Delta^1$ -proline-5-carboxylate reductase (Girija et al., 2002). In the study on sunflower under drought stress conditions, proline increased during stress with increasing gamma-glutamyl kinase activity (Manivannan et al., 2007). Plants show various reactions to Mg deficiency including an increase in proline in the plant. Magnesium deficiency causes plant damage and it is assumed that the availability of sufficient Mg to achieve optimal performance is an effective factor in drought stress conditions. In two other studies, Taha (2016) and Howladav et al. (2014) reported an increase in proline content along with Mg consumption. Chitosan increases proline in stressed and non-stressed conditions, which contributes to the better osmotic regulation of the plant. In other words, chitosan acts as an anti-transpiration and prevents water loss from the surface of the leaf. More increase in proline leads to more resistance in a stressful condition. Emami Bistgani et al. (2017) proved the presence of

chitosan in *Thymus daenesis* Gelak by increasing proline content under stress conditions.

Soluble sugars are important osmolytes which increased in response to drought stress. In general, an increase in soluble sugars during drought stress can be attributed to decaying insoluble carbohydrates leading to an increase in soluble sugars, synthesizing these compounds from non-photosynthetic pathways, and stopping growth (Hsiao, 1973). In another study on rice (Mostajeran and Rahimi-Eichi, 2009) and wheat (Johari-Pireivtlou, 2010), the accumulation of soluble sugars was reported in response to drought stress. Magnesium plays a special role in the activation of the ATP-ase enzyme, which provides the energy needed to absorb sucrose. Therefore, its deficiency reduces soluble sugars and increases ROS production. Correspondingly, an increase in soluble sugars content was observed with magnesium application in pea plant (Howladav et al., 2014). In this study, chitosan consumption caused an increase in soluble sugars, which can be explained by the fact that chitosan is a poly  $\beta$ -(1,4)-2-amino-2-deoxy-D-glucose which degrades to ammonia, an oligosaccharide, and a monosaccharide, leading to an increase in the soluble sugar under foliar application of chitosan. Boonlertnirun et al. (2014) and Saif Eldeen et al. (2014) revealed that chitosan foliar application can increase soluble sugars.

It seems that reducing the protein content under drought stress is related to the protein's reaction with free radicals which results in altering the amino acid, increasing the activity of protein degrading enzymes, reducing protein synthesis, and accumulating free amino acids including proline. In this study, Dashtestan-2 Cultivar had the highest soluble protein content, indicating a relative tolerance compared to Oltan Cultivar. In another experiment by Roy-Macauley et al. (1992), a direct relationship was found between the amount of total protein reduction, the activity of protein degrading enzymes, and the susceptibility of plants to drought stress in bean leaf. They reported that in the susceptible variety of beans, reducing the amount of soluble protein due to drought stress was more than that in the drought tolerant cultivar. However, the activity of

destroying protein enzymes in the sensitive cultivar is more than the resistant one. Magnesium plays an important role in protein synthesis. A large number of studies on protein synthesis have shown that magnesium can enhance the binding of amino acids to tRNA and separate the rhizobium polypeptide chain (Moore and Steitz, 2011). In some cases, a reduction occurs in the amount of protein in Mg deficiency. Based on the results, Mg plays a positive role in protein synthesis, and its lack may weaken the enzymatic synthesis. In this regard, according to Polle et al. (1994), the protein content of the plant decreases under the condition of Mg deficiency. Saad and EL-Kholy (2000) argued that magnesium sulfate foliar application causes an increase in the protein content of the plant. In addition, chitosan leads to an increase in the amount of protein in the plant by increasing the amount of soluble sugars, as well as the activity of the proteinase enzyme. The role of chitosan on increasing ionic content may influence cell membrane stability during an increase in antioxidants and the maintenance of cell membrane from oxidative stress, which results in improved plant growth. Furthermore, foliar application of chitosan was reported to increase the amount of protein in the artichoke plant, compared to the control samples (Saif Eldeen et al., 2014). Also, in another study, the effect of chitosan was reported on the amount of soluble protein (Kumar et al., 2011).

Sesame farming is very important in an arid and semi-arid area like Iran, middle-east, and Africa due to high toleration of sesame in coping with drought and consequently high irrigation efficiency. The results of the present research indicated that photosynthetic pigments decreased with increasing drought intensity due to irrigation cut-off although an increase occurred in the proline content and soluble sugar. These mechanisms indicate that the relative tolerance of sesame against drought is probably high. Of the two cultivars, Dashtestan-2 had higher levels of chlorophyll b, protein, proline, and soluble sugars compared to Oltan. Drought stress induces oxidative stress in the plant and chitosan and Mg-nano foliar application can decrease the resulting damages through osmotic regulators like proline and soluble sugar. Based on the results, it is recommended to use nanofertilizers in order to



improve the physiological characteristics of sesame, as well as reducing the use of common chemical fertilizers, which may result in decreasing the contaminant level in the environment. In addition, Mg and chitosan, improve plant growth and increase plant tolerance under stress conditions by maintaining and enhancing photosynthetic pigments. Positive responses of sesame to chitosan and Mg-nano fertilizer, especially under severe drought stress (irrigation up to 65 BBCH) can be promising for the possibility of stable production of sesame in the arid area. Therefore, supplementary studies may be able to support the idea that application of these two compounds under drought stress and in similar climatic conditions is beneficial in sesame farming.

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## پاسخ‌های فیزیولوژیکی کنجد (*Sesamum indicum* L.) به محلول‌پاشی کودهای کیتوزان و نانو منیزیم تحت قطع آبیاری در سیستم کشاورزی پایدار

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### چکیده فارسی

به منظور بررسی کودهای کیتوزان و نانو منیزیم بر رنگدانه‌های فتوسنتزی، پروتئین، پرولین و محتوی قند محلول کنجد تحت شرایط قطع آبیاری آزمایشی به صورت اسپیلت فاکتوریل بر مبنای بلوک‌های کامل تصادفی با سه تکرار انجام شد. قطع آبیاری براساس مقیاس BBCH به عنوان فاکتور اصلی (آبیاری معمولی، قطع آبیاری در مراحل ۶۵ و BBCH۷۵)، فاکتور دوم به عنوان فاکتورهای فرعی شامل ارقام کنجد اولتان و دشتستان ۲ و محلول‌پاشی نانو منیزیم (محلول‌پاشی و عدم محلول‌پاشی) و کیتوزان (شاهد، محلول‌پاشی  $4/8 \text{ g.L}^{-1}$  در مرحله BBCH ۶۵ و  $6/4 \text{ g.L}^{-1}$  در مرحله BBCH ۷۵). طبق نتایج بدست آمده، تنش شدید خشکی (آبیاری تا مرحله BBCH ۶۵) منجر به کاهش میانگین صفات کلروفیل a، b و کل در مقایسه با تیمار شاهد گردید. محلول‌پاشی کیتوزان در مقایسه با نانومینیزیم نتایج مطلوب‌تری نشان داد و باعث افزایش میانگین صفات کلروفیل a، b، کل، کاروتنوئید، پروتئین، پرولین و قندهای محلول گردید. علاوه بر این، آبیاری تا مرحله BBCH ۶۵ (تنش شدید خشکی) و عدم محلول‌پاشی نانو منیزیم منجر به کاهش محتوی کلروفیل و خسارت فیزیولوژی گردید. براساس این یافته‌ها، پلیمر زیستی کیتوزان به عنوان یک ماده طبیعی و از طرفی با توجه به نقش عنصر منیزیم در ساختار کلروفیل و بسیاری از آنزیم‌های حیاتی گیاه، محلول‌پاشی این دو می‌تواند راه کار مناسبی جهت کاهش خسارت گیاهان در شرایط تنش خشکی باشد.

**کلمات کلیدی:** تنش خشکی، تنظیم اسمزی، رنگدانه‌های فتوسنتزی، عملکرد مواد مغذی، مقیاس BBCH