Physiological responses of two tomato (*Lycopersicun esculentum* M.) cultivars to Azomite fertilizer under drought stress

Hamid Noorani Azad*[1], Abolghasem Hassan Poor[2], Gholam Reza Bakhshikhaniki[1] and Mohammad Ali Ebrahimi[3]

1. Department of Biology, Payame Noor University , P.O.Box 19395-1697, Tehran, Iran.
2. Iranian Research Institute of Plant Protection, Tehran, Iran.
3. Department of Agriculture Biotechnology, Payame Noor University, P.O.Box 19395-1697, Tehran, Iran.

Abstract

This study was conducted in order to investigate the effect of drought stress and Azomite fertilizer on some physiological traits of two tomato (*Lycopersicon esculentum* M.) cultivars (izmir and Izabella). A randomized complete design with factorial arrangement with three replications was used. Treatments consisted of three levels of irrigation including FC (control), $\frac{2}{3}$ FC (mild drought stress), and $\frac{1}{3}$ FC (severe drought stress)] along with four levels of Azomite (0, 25, 50 and 100g/pot). Results showed that drought stress reduced stem length, plant dry and fresh biomass, relative growth rate (RGR), net assimilation rate (NAR), relative water content (RWC), total chlorophyll, carotenoid, nitrogen, phosphorus and potassium in leaves. Azomite fertilizer increased the stem length, plant dry and fresh biomass, RGR, NAR, RWC, total chlorophyll, carotenoid, nitrogen, phosphorus, and potassium in leaves in comparison with control plants in both cultivars. Interaction effect of drought stress and Azomite had a significant effect on increasing plant fresh biomass, RGR, NAR, RWC, total chlorophyll, carotenoid, nitrogen, phosphorus, and potassium. Interaction effect of drought stress and cultivar showed significant effect on increasing plant fresh biomass, NAR, RWC, total chlorophyll, and phosphorus. Moreover, the results indicated that the interaction effect of Azomite and cultivar had a significant effect on plant fresh biomass, RGR, RWC, and phosphorus in leaves. In general, Azomite was effective on drought stress tolerance of tomato plant.

Key words: *Lycopersicon esculentum*; azomite; drought stress; chlorophyll; RWC

Abbreviations

FC: field capacity; NAR: net assimilation rate; RGR: relative growth rate; RWC: relative water content

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Introduction

Studies show that water shortage delays growth and development in plant, reduces leaf size, and causes anatomical changes due to alteration in cell size, senescence, and ultimately

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*Corresponding author
E-mail address: noorani320@gmail.com

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death in many plant species (Jaleel *et al.*, 2008). Reduction in water absorption in drought stress conditions decreases intracellular water and turgidity pressure, which subsequently limits cell division and development through mitosis and reduces growth (Osakabe *et al.*, 2004). One of the most accurate ways to study plant reactions to environmental conditions is through evaluation of physiological growth indicators (Karimi and Siddique, 1991). Relative Growth Rate (RGR) is a good indicator to present plants with improvement capability against detrimental effects of drought stress (Xu *et al.*, 2009). Studies show that drought stress reduces relative growth rate in tomato (Sanchez-rodriguez *et al.*, 2010). Moreover, drought stress reduces nutrient absorption and subsequently decreases cell growth and development, leaf development, and biomaterials' absorption, composition, and transfer in plants. Drought stress also reduces root capacity to absorb water and nutrients from soil due to reducing nutrient absorption in plants (Osakabe *et al.*, 2014).

Nitrogen is an important element in providing carbon skeleton and producing metabolites and enzymes and nitrogen shortage reduces plant growth in drought stress conditions (Singh *et al.*, 2005). Phosphorous shortage alters water absorption in plant and largely reduces stomata conductive and subsequently photosynthesis and growth (Farooq *et al.*, 2009). It has been shown that decreasing relative water content may reduce stomata conductive, photosynthesis, CO_2 production, and plant growth in drought stress (Cornic and Fresneau, 2002).

Sanchez-Rodrugueze in 2010 studied genotypes in tomato and reported a positive relationship between relative growth rate in the plant and relative water content in leaf. Water shortage in plant environment damages pigments and plastids and reduces chlorophyll and carotenoid in most plants (Fellows and Boyer, 1996). In addition to prevention of water absorption, different nutrients absorption may also be limited in drought stress conditions. Proper nutrition is known as one of the plant production management mechanisms in different environmental conditions (Wariach *et al.*, 2011).

Biological fertilizers and natural inputs in ecosystems are one of the main factors in sustainable agriculture in order to eliminate or reduce chemical elements (Patel *et al.*, 2011). Azomite is an inorganic, 100% natural compound without any additional elements, which has been used in organic agriculture because it is not synthetic and does not have any environmental pollution (Yarrow, 2000).

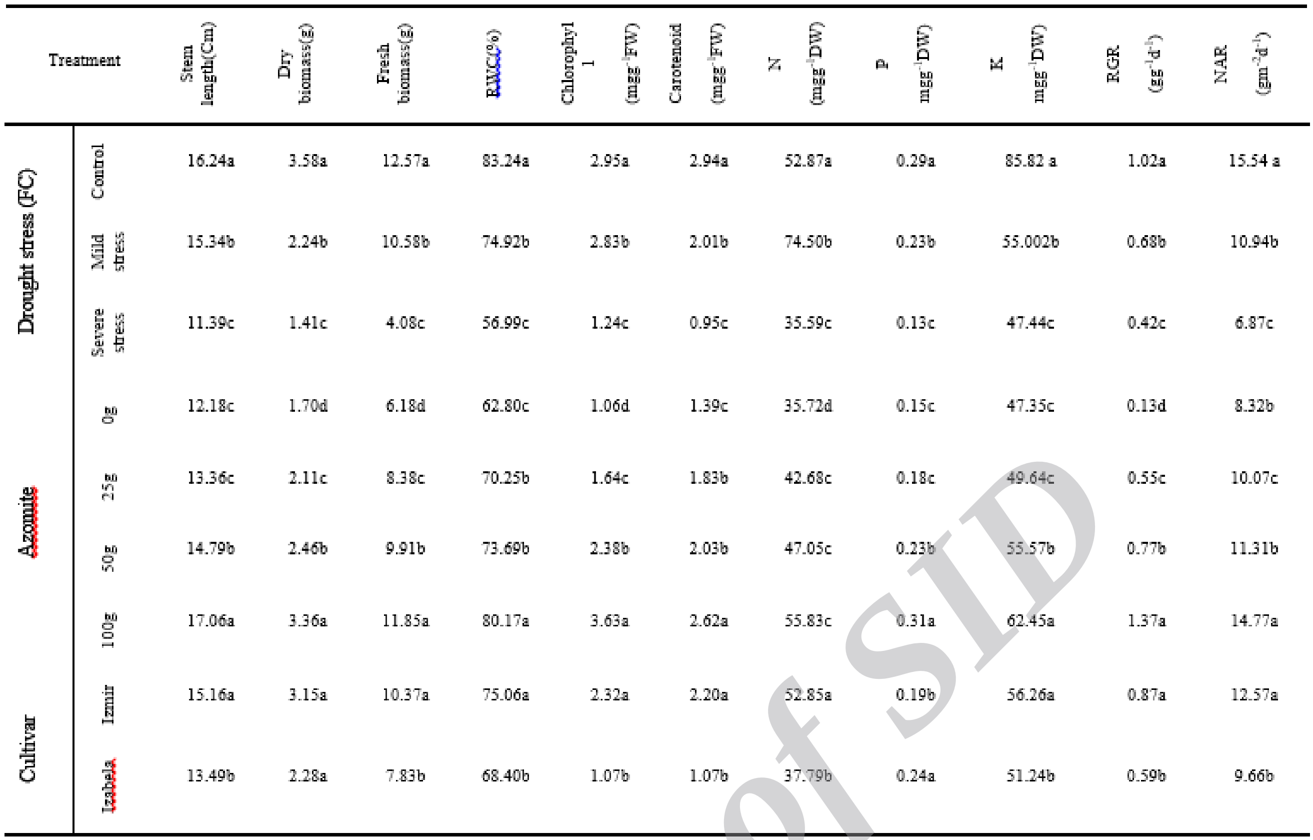
Tomato (*Lycopersicun escluentum* M.), belongs to Solanaceae family and is largely cultivated in different regions especially warm and semidry climates. Irrigation and proper nutrition are environmental factors, which affect production and function of this plant (Wang *et al.*, 2011). The aim of this study was investigating the effects of drought stress and azomite fertilizer on biomass, some of physiological growth indices, chlorophyll, carotenoid, relative water content, nitrogen, phosphorus and potassium content in two tomato cultivars, namely, Izmir and Izabella under greenhouse condition.

Materials and Methods

Plant material and growth conditions

The seeds of two tomato cultivars, Izmir and Izabella, were obtained from Seed and Plant Improvement Institute, Karaj, Iran. The seeds of these cultivars were germinated and grown for 30 days in individual pots (25 cm upper diameter, 17 cm lower diameter, and 25 cm height) and filled with sand, clay, and silt (2:1:1), the soil pH was maintained at about 7.6. All pots were kept in greenhouse under controlled conditions with relative humidity of 65%, at 25±1 °C - 15±1 °C (day/night), and a 16 h/8 h photoperiod with a photosynthetic photon-flux density of 450 µmol $m^{-2}s^{-1}$ (measured with an SB quantum 190 sensor, LICOR Inc., Lincoln, NE, USA). Irrigation was done thrice a week according to soil FC for one month. The water stress and Azomite fertilizer treatments began 30 days after germination and maintained for 25 days. The first group, i.e., control was irrigated according to FC, the second group received mild stress (⅔ FC), and the third group received severe stress (½FC). The control plants received 300 ml water and the mild and severe drought stress treatments involved 200 ml and 100 ml water every 3 days, respectively. Four treatments were tested for Azomite, included the control (0 g/pot), 25, 50, and 100 g Azomite at per pot.

Table 1. Mean comparison of traits of two tomato varieties under drought stress treated with azomite



Treatment		Stem length(Cm)	Dry biomass(g)	Fresh biomass(g)	RWC(%)	Chlorophyll (mgg^{-1}FW)	Carotenoid (mgg^{-1}FW)	N (mgg^{-1}DW)	P mgg^{-1}DW)	K mgg^{-1}DW)	RGR (gg^{-1}d^{-1})	NAR (gm^{-2}d^{-1})
Drought stress (FC)	Control	16.24a	3.58a	12.57a	83.24a	2.95a	2.94a	52.87a	0.29a	85.82 a	1.02a	15.54 a
	Mild stress	15.34b	2.24b	10.58b	74.92b	2.83b	2.01b	74.50b	0.23b	55.002b	0.68b	10.94b
	Severe stress	11.39c	1.41c	4.08c	56.99c	1.24c	0.95c	35.59c	0.13c	47.44c	0.42c	6.87c
Azomite	0g	12.18c	1.70d	6.18d	62.80c	1.06d	1.39c	35.72d	0.15c	47.35c	0.13d	8.32b
	25g	13.36c	2.11c	8.38c	70.25b	1.64c	1.83b	42.68c	0.18c	49.64c	0.55c	10.07c
	50g	14.79b	2.46b	9.91b	73.69b	2.38b	2.03b	47.05c	0.23b	55.57b	0.77b	11.31b
	100g	17.06a	3.36a	11.85a	80.17a	3.63a	2.62a	55.83c	0.31a	62.45a	1.37a	14.77a
Cultivar	Izmir	15.16a	3.15a	10.37a	75.06a	2.32a	2.20a	52.85a	0.19b	56.26a	0.87a	12.57a
	Izabela	13.49b	2.28a	7.83b	68.40b	1.07b	1.07b	37.79b	0.24a	51.24b	0.59b	9.66b

The similar letters in every column for each individual show an not significant amount ($p<0.05$).

Estimation of the shoot and root biomassand height

Four replicates of the control and treated plants (four plants in each) were harvested and the shoots and roots of each plant were collected separately for estimation of shoot and root fresh and dry weight and height. The shoot and root biomass and height were expressed as g plant^{-1} and cm shoot and root^{-1}, respectively.

Estimation of growth analysis

Three-week-old seedlings were harvested for RGR and NAR calculation before treatments (day 0). After the treatments, plants were randomly selected for the growth analyses and were separated to shoot and root fractions. Shoots were dried at 70 °C for 72h and dry weights were used to calculate the RGR and NAR of shoots according to the method of Hunt *et al.* (2002).

Chlorophyll and carotenoid contentswere determined using Lichtenthaler method (1987). 0.05 g of fresh leaf was extracted in 10 ml 80% acetone (v/v). The absorbance of the extracts were then measured at 663, 645, and 470 nm for chlorophyll a, b, and carotenoid using a UV/visible spectrophotometer (UnicamUV-330, USA). Chlorophyll and carotenoid contents were estimated based on mgg^{-1} FW.

Determination of the relative water content (RWC)

Third leaves (n=6) were obtained from each treatment group and their fresh weight (FW) was determined. The leaves were floated on deionized water for 6 h under low irradiance and then the turgid tissue was quickly blotted to remove excess water and their turgid weights (TW) were determined. Dry weight (DW) was determined after the leaves were dried in the oven. RWC was calculated by the following formula (Barrs and Freshherley, 1962):

Table 2.
Comparison of the mean interaction effects of irrigation and Azomite on the traits measured

irrigation	azomite	Stem length(Cm)	Dry biomass(g)	Fresh biomass(g)	RWC(%)	Chlorophyll (mgg^{-1} FW)	Carotenoid (mgg^{-1} FW)	N (mgg^{-1} DW)	P (mgg^{-1} DW)	K (mgg^{-1} DW)	RGR ($gg^{-1}d^{-1}$)	NAR ($gm^{-2}d^{-1}$)
control	0g	14.26c	2.66cd	9.51d	81.76a	1.63b	83.25a	45.57c	0.191d	43.29c	0.21e	13.95b
	25g	15.49bc	3.05c	11.86c	82.26a	2.64bc	78.81ab	47.40bc	0.235cd	54.90b	0.85c	13.50b
	50g	16.53b	3.18b	13.66b	83.43a	3.01b	63.11c	55.21b	0.349b	65.69a	1.21b	15.36b
	100g	18.68a	4.79a	15.28a	85.68a	3.78a	63.11c	63.31a	0.390a	71.39a	1.59a	19.36a
mild stress	0g	12.79cd	1.53f	7.12e	67.71b	1.10ef	83.31a	35.69d	0.150de	43.40c	0.12e	7.62c
	25g	14.82c	1.89ef	9.68d	74.17b	2.05c	71.03b	46.58bc	0.207d	42.89c	0.49d	9.90c
	50g	16.63b	2.39d	11.80c	75.68ab	2.13c	50.87d	52.95d	0.259c	48.67bc	0.91c	10.68c
	100g	17.82ab	3.15c	13.76b	82.11a	2.78bc	50.87d	54.77d	0.335b	54.78b	1.21b	15.55b
severe stress	0g	9.21e	0.91g	1.94h	38.95e	0.74f	83.31a	26.91e	0.118e	55.37b	0.77e	3.40e
	25g	10.52de	1.39f	3.60gh	54.31d	0.81f	71.03b	32.98de	0.108e	52.34b	0.29d	6.81d
	50g	11.21d	1.21fg	4.26g	61.97cd	0.96f	50.87d	34.07de	0.110e	51.24b	0.30d	7.89d
	100g	14.63b	2.14d	6.51f	72.73b	1.28ef	50.87d	42.43bc	0.218d	61.17ab	0.51c	9.40cd

The similar letters in every column for each individual show an not significant amount ($p<0.05$).

RWC=(FW - DW)/(TW - DW)×100

Determination of the mineral oncentrations

Mineral concentrations were measuredin dried leaves. Nitrogen was determined using the micro Kjeldahl method as described by AACC (2000); phosphorus was determined by spectrophotometer method as described by Snell and Snell (1954) and potassium was estimated using flame photometer method described by Chapman and Pratt (1978).

Statistical Analysis

All experiments were performed with 4 replications using a completely randomized design. Data were statistically analyzed by one-way analysis of variance using SAS and the means were compared by Duncan's multiple range testat 0.05 probability level.

Results

The results showed that the rate of all studied traits significantly reduced compared to control in drought stress condition. Increasing Azomite level significantly elevated fresh and dry weight, RWC, total chlorophyll, carotenoid, nitrogen, RGR, NAR, and increased stem length, phosphorous, and potassium in leaf except for25g Azomite treatment as compared with control. All the studied traits showed higher rate in Izmir compared with Izabella except phosphorous level in leaf and these higher rates were significantinall studied traits except dry weight in plant (Table 1).

The comparison of the effect of drought stress and Azomite on the studied traits showed the highest rate in the treatment without drought stress and with 100g Azomite and the lowest rate in the treatment with severe drought stress and without Azomite (control). Each trait showed significant changes. In mild stress, increasing Azomite level elevated all the studied traitsexcept

Table 3
The interaction of drought stress and cultivar on the studied traits

Cultivar	drought stress	Stem length(Cm)	Dry biomass(g)	Fresh biomass(g)	RWC(%)	Chlorophyll (mgg^{-1}FW)	Carotenoid (mgg^{-1}FW)	N (mgg^{-1}DW)	P (mgg^{-1}DW)	K (mgg^{-1}DW)	RGR (gg^{-1}d^{-1})	NAR (gm^{-2}d^{-1})
Izmir	Control	17.3a	3.457a	13.535 a	83.25a	3.066a	3.251a	60.620a	0.249b	61.529 a	0.898a	17.763 a
	mild stress	15.8ab	2.369b	10.013b	78.81ab	2.725a	2.229bc	54.117ab	0.213b	55.058b	0.618b	12.178b
	severe stress	12.2c	1.368c	5.452d	63.11c	1.181c	1.133d	43.795b	0.125c	52.196b	0.377c	8.791d
Izabela	Control	15.1b	3.702a	11.623b	83.31a	3.843a	2.635b	45.095b	0.332a	56.116b	1.599a	14.335b
	mild stress	14.7b	2.120b	9.173c	71.03b	1.962b	1.807c	40.885b	0.263b	54.946b	0.752b	9.707cd
	severe stress	10.5c	1.465c	2.711e	50.87d	1.303c	0.784d	27.400c	0.152c	43.683ab	0.246c	2.96e

Similar letters in every column show the differences are not significant ($p<0.05$).

potassium in leaf in comparison with severe drought stress (Table 2).

The relationship between drought stress and cultivar showed that decreases in other traits' rate along with the increase in drought stress in Izabella is higher than Izmir except for stem length and carotenoid in leaf compared to control in each cultivar (Table 3).

The relationship between Azomite and cultivar showed that increasing stem length, carotenoid, phosphorous, potassium, RGR, and NAR along with increasing Azomite is higher in Izabella compared with Izmir and both cultivars show improvements in these traits in comparison with control while the increase in fresh and dry weight, RWC, total chlorophyll, and nitrogen in leaf was higher in Izmir than Izabella (Table 4).

Discussion

The results showed that increasing drought stress decreased stem length, dry and fresh weight, RGR, and NAR in both cultivars. Izabella cultivar has shown higher decrease in the studied traits than Izmir cultivar compared to the control in each cultivar. Reduction in water absorption in drought stress conditions decreases intracellular water and turgidity pressure, which subsequently inhibits cell division and development and reduces growth and dry mass storage (Delfine *et al.*, 2002). Sanchez-Rodriguez *et al.* (2010) reported a decrease in biomass and RGR in tomato plant in drought stress conditions. Sekmen *et al.* (2014) showed a decrease in NAR, RGR, and dry biomass in cotton plant due to a decrease in leaf area, chlorophyll and photosynthesis and an increase in respiration in drought stress conditions. In the current study, a decrease in RGR rate in Izabella cultivar (84.6%) in comparison with Izmir cultivar (62.5%) in drought stress may indicate that Izmir cultivar is more resistant to drought stress than Izabella cultivar. A decrease in fresh biomass in drought stress could stop growth and development of cells due to a decrease in turgidity pressure (Sankar *et al.*, 2007). In the current study, a higher decrease in fresh biomass in Izabella cultivar (76.6%) compared with Izmir cultivar (59.7%) in drought stress may indicate that Izmir cultivar is more tolerant to drought stress than Izabella cultivar. Higher water conservation capacity in drought stress condition is an important way for adaptation and resistance

Table 4
The interaction of Azomite and cultivar on the studied traits

Cultivar	azomite	Stem length(Cm)	Dry biomass(g)	Fresh biomass(g)	RWC(%)	Chlorophyll (mgg^{-1}FW)	Carotenoid (mgg^{-1}FW)	N (mgg^{-1}DW)	P (mgg^{-1}DW)	K (mgg^{-1}DW)	RGR (gg^{-1}d^{-1})	NAR (gm^{-2}d^{-1})
Izmir	Control	12.7 cd	1.588 c	6.213 d	64.51 b	1.002 d	1.679 bc	41.917 bc	0.125 e	51.965 b	0.133 e	9.705 c
	25g	14.7 b	2.125 bc	9.710 c	76.87 a	1.953 d	2.152 b	51.629 ab	0.157e	53.913 b	0.556 D	11.625 bc
	50g	15.8 ab	2.441 b	11.335 d	76.31 a	2.521 b	2.294b	54.708 ab	0.215 c	56.665 ab	0.780 c	13.121 b
	100g	17.1 a	3.439 a	14.059 a	82.53 a	3.819 a	2.695 a	63.173 a	0.285 b	62.501 a	1.185b	14.759 a
Izabela	Control	11.4 d	1.825 c	6.159 d	61.101 b	1.119 d	1.114 d	29.535 c	0.180 d	42.745 c	0.130 e	6.943 d
	25g	11.8 b	2.103 bc	7.053 d	63.62 b	1.336 d	1.524 c	33.742 c	0.209 de	45.369 c	0.545 d	8.524 cd
	50g	13.6 b	2.493 b	8.485 c	71.08 ab	2.238 b	1.778 bc	39.393 bc	0.264 bc	54.479 b	0.773 c	9.508 c
	100g	16.9 a	3.294 a	9.648 bc	77.81 a	3.452 a	2.552 a	48.503 b	0.353 a	62.404 a	1.566 a	13.695 b

Similar letters in every column show that the difference is not significant ($p<0.05$).

(Selote and Chopra, 2002). RWC is a reliable factor to show hydration rate in plant cells (Sanchez-Rodriguez et al., 2010). Rampino *et al.* (2006) showed that plant ability to maintain cellular water is one of the most important survival factors in drought stress condition and susceptible and resistant cultivars in wheat can be differentiated based on RWC.

The results showed that using Azomite fertilizer increased stem length, fresh and dry biomass, and RGR and NAR in both cultivars compared to the control. Azomite is a fertilizerfull of soluble nutrients in water, which increases auxin level in plant and develops plant roots and absorption of organic elements and water (Palmer and Sharon, 2009). Azomite may increase soil nutrients and its absorbance and thus enhance growth in both cultivars. In this study, increasing Azomite enhanced RWC in both cultivars (Table1). Using Azomite increases water content in leaf due to potassium level. Palmer and Sharon (2009) reported that Azomite is considered a natural material as it contains 5% potassium, almost 3% calcium and 1% magnesium. Potassium is an osmotic material, which plays a role in maintaining turgidity pressure and water absorption (Saeedakram *et al.*, 2009). The results showed that increasing drought stress decreases total chlorophyll and carotenoid level in both cultivars. Our results are consistent with those of Ghorbanli *et al.* (2013) on tomato. Thalooth *et al.* (2006) reported that in drought stress condition, chlorophyllase and peroxidase functionsincreased and chlorophyll destroyed more than its synthesis and therefore, chlorophyll level decreased. Carotenoids play a protection role against induced oxidative stress and thus are destroyed (Schutz and Fangmeir, 2001). A decrease in chlorophyll reduces photosynthesis and photosynthetic products and subsequently reduces growth. The current study showed that increasing Azomite enhanced photosynthetic pigments in both. Azomite contains lots of dioxy silicon (Palmer and Sharon, 2009). Using silicon in corn culture under salinity stress condition increases chlorophyll a and b content and therefore increases membrane permeability and photosynthesis level (Osakabe *et al.*, 2014). The results showed that drought stress decreased nitrogen, phosphorous, and potassium level in both cultivars. It has been shown that in drought stress, absorption and accumulation of N and K elements in cotton shoots significantly decreased (McWilliams, 2003). Lack of phosphorous element in shoots of bean genotypes in drought stress might be due to insignificant movement of phosphate ion (Peuke and Remember, 2004). The relationship between drought stress and Azomite showed that increasing Azomite enhances nitrogen and phosphorous in leaves in mild and severe stress with significant increase in mild stress. Potassium level, however, increased higher in severe stress than mild stress. Azomite contains pentoxide phosphorous and nitrogen with 5% potassium (Palmer and Sharon, 2009). In drought stress conditions, plant roots face lack of water and nutrients such as nitrogen and therefore, nitrogen absorption from soil and its concentration in plant may decrease (Singh et al., 2005). Pinior *et al.* (2005) reported that phosphorous fertilizers increase water consumption yield and plant growth and increasing soil humidity enhances absorption of this element. Decreasing diffusion resistance level of leaf stomata in the sunflowers treated with potassium ion in drought stress condition increases absorption and transport of this ion to plant shoots compared to control (Lindhauer *et al.*, 2007). In this study, Azomite may increase absorption and accumulation of ions in plant leaves due to the presence of K, P, and N. Higher accumulation of potassium ion in severe drought stress in comparison with mild stress could indicate the important role of potassium in osmosis regulation (Thalooth *et al.* 2006).

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واکنشهای فیزیولوژیکی دو رقم گوجه فرنگی(*Lycopersicun esculentum* M.) به کود آزومایت تحت تنش خشکی

حمید نورانی آزاد*[1] ، ابوالقاسم حسن پور[2] ، غلامرضا بخشی خانیکی[1]، محمد علی ابراهیمی[3]

۱. گروه زیست شناسی، دانشگاه پیام نور، ص. پ. ۴۶۹۷-۱۹۳۹۵، تهران، ایران

۲. مرکز تحقیقات گیاهپزشکی کشور، تهران، ایران

۳. گروه بیوتکنولوژی کشاورزی ، دانشگاه پیام نور، ص. پ. ۴۶۹۷-۱۹۳۹۵، تهران، ایران

*مسئول مکاتبات: Noorani320@gmail.com

چکیده فارسی

به منظور بررسی اثرات تنش خشکی و کود آزومایت بر برخی ویژگی های فیزیولوژیکی دو رقم گوجه فرنگی(ازمیر و ایزوبلا)، آزمایشی انجام شد. تیمارهای آزمایش شامل آبیاری در سه سطح (آبیاری بر اساس ۱۰۰درصد ظرفیت مزرعه به عنوان شاهد، $\frac{2}{3}$ و $\frac{1}{3}$ ظرفیت مزرعه)، و چهارسطح آزومایت(صفر، ۲۵، ۵۰ و ۱۰۰گرم در هر گلدان) با سه تکرار به صورت فاکتوریل در قالب طرح کاملا تصادفی بررسی شد. نتایج نشان داد که تنش خشکی سبب کاهش طول ساقه، بیوماس تر و خشک گیاه، میزان رشد نسبی، میزان ماده سازی خالص، محتوای نسبی آب، کلروفیل کل، کاروتنوئید، نیتروژن، فسفر و پتاسیم برگ شد. آزومایت باعث افزایش طول ساقه، بیوماس تر و خشک گیاه، میزان رشد نسبی، میزان ماده سازی خالص، محتوای نسبی آب، کلروفیل کل، کاروتنوئید، نیتروژن، فسفر و پتاسیم برگ در مقایسه با گیاهان شاهد در هر دو رقم شد. اثر متقابل تنش خشکی و آزومایت بر افزایش بیوماس ترگیاه، میزان رشد نسبی، میزان ماده سازی خالص، محتوای نسبی آب، کلروفیل کل، کاروتنوئید، نیتروژن، فسفر و پتاسیم برگ معنی دار بود. اثر متقابل تنش خشکی و رقم تاثیر معنی دار بر افزایش بیوماس تر گیاه، میزان ماده سازی خالص، محتوای نسبی آب، کلروفیل کل و فسفربرگ داشت. اثر متقابل آزومایت و رقم تاثیر معنی دار بربیوماس تر گیاه، میزان رشد نسبی، محتوای نسبی آب، کلروفیل کل و فسفر برگ نشان داد. بطور کلی آزومایت درمقاومت به تنش خشکی گیاه گوجه فرنگی موثربود.

کلمات کلیدی: *Lycopersicon esculentum*، آزومایت، تنش خشکی، کلروفیل، محتوای نسبی آب