

THE INFLUENCE OF SILICA UPON QUANTITATIVE, QUALITATIVE, AND BIOCHEMICAL TRAITS OF TOMATO UNDER WATER STRESS

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ABSTRACT

Water stress is by far the most serious limiting factor to tomato (*Solanum lycopersicom*) production, particularly in Iran where located in arid and semi-arid regions. Silicon (Si) is considered an effective element to mitigate the adverse effects of water stress by promoting plant growth and production. Therefore, the present study was designed to evaluate the effects of the foliar application of Si (0, 100, and 200 mg L⁻¹) and three water regimes – no stress (100), mild stress (80%), and severe stress (60%) – on the growth parameters, the yield, and the fruit quality as well as antioxidant status of the tomato. The imposed water stress significantly increased the total soluble solids (TSS), the total acidity (TA), and the flavonoids as well as antioxidant defense parameters such as catalase (CAT) and peroxidase (POX), while the growth parameters (plant height and leaf number) and tomato yield were decreased. In contrast, the foliar application of Si (200 mg L⁻¹) remarkably improved the total yield of tomatoes when exposed to water stress by improving the antioxidant enzyme activities and total flavonoid compounds. In addition, the application of Si could significantly improve the growth parameters (plant height and leaf number) and fruit quality (fruit firmness and size). As a result, the foliar application of Si could be suggested as an effective strategy for imparting water stress resistance in the tomato.

Key words: water stress, silicon, tomato yield, growth parameters, antioxidant parameters

INTRODUCTION

Water stress is one of the main stress factors responsible for limiting agricultural productivity [Fahad et al. 2017]. This abiotic stress is an important threat to constrain crop growth leading to yield reduction and smaller fruit size in different agricultural crops including wheat [Maghsoudi et al. 2019], eggplant [Çolak et al. 2015], and pepper [Kaya et al. 2019]. Severe water stress could restrict photosynthesis rate, plant growth [Verma et al. 2019], tissue water potential,

and cell membrane stability in plants [Kapoor et al. 2020]. Moreover, water limitations cause changes in the hormonal balance due to altering the plant metabolism which results in increased generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [Sharma et al. 2020]. ROS and RNS including free radicals such as H₂O₂ and ·OH can disrupt normal cell metabolism by causing peroxidation of lipids, oxidation of proteins, and damage to nucleic acids [Shar-

ma et al. 2012]. However, plants are naturally able to tolerate water stress through a mechanism of defense induction and altering the cell metabolism [Basu et al. 2016]. This mechanism includes enzymatic and non-enzymatic antioxidant defense systems in order to protect the cell against oxidative stress [Zhen et al. 2012, Gharibi et al. 2016]. Previous studies on different plant species such as *Hibiscus sabdarrifa* L. [Ali and Hassan 2017] and *Arachis hypogaea* L. [Shinde et al. 2018] have shown an increase in antioxidant activities; superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GPx), and ascorbate peroxidase (APX) in response to water deficit conditions [Cao et al. 2017, Bhatnagar-Mathur et al. 2009]. In terms of non-enzymatic defense mechanisms, increasing the concentration of ascorbic acid and phenolic compounds makes plants more resistant to water stress as well [Farooq et al. 2020].

Tomato (*Solanum lycopersicom* [syn: *Lycopersicon esculentum* Mill.]), belonging to the Solanaceae family, is the most popular vegetable worldwide due to the rich source of minerals, vitamins, and antioxidants [Dehgan et al. 2014, Wakil et al. 2017]. Although it can be cultivated in most countries of the world except cold regions, water deficit is still the most important abiotic factor restricting its growth and production [Xiukang and Yingying 2016]. Particularly in Iran, water shortage is found the principal limiting factor responsible for decreasing tomato production because more than 75% of Iran's total area is located in arid and semi-arid regions [Madani 2014]. As a result, the maintenance of proper nutrition to plants has been found critical to reducing the negative impacts of water scarcity on crop yield. Silicon (Si), the second most abundant element in the earth's crust, is a very good example. Although it is not as an essential plant nutrient as other known elements like phosphorus (P), calcium (Ca), and nitrogen (N) for plant growth [Zhang et al. 2017], it has gained considerable attention due to enhancing plant tolerance to environmental stresses, particularly water stress [De Camargo et al. 2019, Kumar et al. 2020]. Plants' roots commonly absorb the Si in the form of monosilicic acid or orthosilicic acid (H_4SiO_4) [Tubaña and Heckman 2015]. The major mechanisms of Si-mediated alleviation of water stress in different

plant species include activation of an antioxidant defense system, prevention of transpirational water loss [Agarie et al. 1998], enhancing water uptake by roots [Sonobe et al. 2010, Liu et al. 2014], stimulating osmolyte accumulation [Ming et al. 2012], increasing the activity of the photosynthetic enzyme [Gong and Chen 2012] and regulating growth substance levels [Zhu and Gong 2014]. Different studies demonstrated the positive impact of Si application on the fruit yield and the antioxidant enzyme activities (SOD, APX, and CAT) of diverse plant species subjected to water stress [Zeng et al. 2011].

Due to the importance of water saving in many countries like Iran, the use of Si fertilizer could be an effective way to mitigate the undesirable effects of water stress on tomato yield. Therefore, the aim of this work was to evaluate the influence of water limitations (60 and 80 of the crop water requirement) and exogenous Si (0, 100, and 200 mg L⁻¹) applied to the quantitative, qualitative, and biochemical traits of tomato (*Lycopersicon esculentum* L. cv BHN).

MATERIAL AND METHODS

Study site and experimental procedure

The study was carried out in the research greenhouse of the Faculty of Agriculture, University of Guilan, Iran, during the tomato-growing season in 2017 and 2018. Tomato (BHN variety) seeds were cultivated in plastic pots (at 1–2 cm of soil depth) containing coco peat (80%) and perlite (20%). The pots were irrigated daily and kept under the average day temperature of 20 ±5°C and the average night temperature of 15 ±5°C. At the four and five-leave stages, the tomato seedlings were transferred to the main pots (10 L capacity, 24 cm height, and 24 cm internal diameter) containing coco peat and perlite. The pots were irrigated two times a day. Hoagland nutrient solution with electrical conductivity of 2/7 dS m⁻¹ and pH of 6.5 was used to feed the seedlings three times a day. It was used a drip irrigation system for delivering the nutrient solution and its excess was collected in drainage and discarded. At the six-leave stage, a completely randomized design with three replications was conducted with three irrigation levels – 60 (severe stress), 80 (mild stress), and 100% water requirement – and three

levels of Si treatment (0, 100, and 200 mg L⁻¹). Silicic acid (Silamol®) was used as the silicon source and was sprayed onto the leaves of the appropriate plants at vegetative and flowering stages in the morning. These chemicals were sprayed for three consecutive days to ensure their uptake by the plants. The chemical accumulation on the soil surface was prevented by maintaining the drainage of the pots at 20–25% while an adequate intake of nutrients was absorbed by the plants. The pots were irrigated three times per day and took 30 min, 20 min and 10 min for groups under no water stress, mild water stress, and severe water stress each time.

In each stage, the water requirements of tomatoes were calculated based on the potential evapotranspiration, irrigation interval, and crop coefficient. An evaporation pan installed in the greenhouse was used to estimate the potential evapotranspiration. After that, the irrigation depth was calculated using the crop coefficient [Allen et al. 1998].

Growth characters and fruit quality parameters. Five plants were randomly taken from each experimental plot to measure the plant height and the leaf number as well as the number of fruits, the fruit weight, and tomato yield. The tomato diameter and thickness were measured using a caliper. The TSS and TA were determined using the method of Zomorodi et al. [2006]. Contents of vitamin C and the fruit firmness proline were measured following Rangana [1979] and Kader et al. [1978], respectively.

Antioxidant capacity, total flavonoids, and antioxidant enzyme activities. The antioxidant capacity of the fruit extracts was estimated by the DPPH method [Re et al. 1999]. The concentration of the total flavonoids was calculated using a spectrophotometer (506 nm) [Kaneda et al. 2006]. The antioxidant enzyme activities (CAT and POX) were measured by spectrophotometer at 20 nm and 470 nm (JENWAY spectrophotometer model UV-6505) [Cakmak and Horst 1991, Ghanati et al. 2002].

Fruit nitrogen, potassium, and Si contents. Determination of N by using Kjeldahl's method [Ostrowska et al. 1991], measurement of P by using flame photometry [AbdelShafy et al. 1994], and Si was determined by spectrophotometer (Varian, Kerry 100) at 650 nm [Moyer et al. 2008].

Data analysis. The data were analyzed by Statistical Analysis Software (Ver. 9.1, SAS Inc., Cary, NC) and the comparison of the means was performed based on the LSD test ($P \leq 0.01$).

RESULTS

Effect of water deficit and silicon on plant growth and fruit quality parameters

The plant height was significantly ($P < 0.01$) affected by the water deficit. As there was a 20% reduction in the tomato plant height under the severe water stress (60%) condition (Fig. 1A). The leaf number was also similarly influenced (Fig. 1B) by the severe stress as it decreased by 33% ($P < 0.05$). Exogenously applied Si at 100 and 200 mg L⁻¹ doses alone or in combination with the irrigation treatments did not affect the plant height of the tomato, whereas the leaf number was significantly ($P < 0.05$) increased in the groups subjected to the 200 mg L⁻¹ Si by about 30% (Fig. 2A) (Tab. 1).

Severe and mild water stress remarkably ($P < 0.01$) reduced the fruit number by about 65% and 25% respectively. Similarly, the water deficit caused a significant ($P < 0.01$) decrease in the fruit weight (Fig. 1D) and fruit size (Figs. 1E, and 1F). The present study also showed that the administration of 200 mg L⁻¹ Si ($P < 0.01$) could improve the fruit number by approximately 65% as compared to the non-treated group (Fig. 2B). A 50 percent increase in fruit weight was found in the group treated by 200 mg L⁻¹ Si (Fig. 2C). The fruit size was positively influenced by the foliar application of 200 mg L⁻¹ (Figs. 2D–2E) as compared to the control group.

Thus, the heaviest and biggest fruit was found in the groups treated with full irrigation (100%) and 200 mg L⁻¹ Si. Tomato yield decreased significantly under water stress by above 25%. However, the foliar application of Si caused a significant increase in tomato yield under water-limited conditions (Fig. 5B).

The water stress increased significantly ($P < 0.01$) TSS (Fig. 4A) by 50% but decreased vitamin C by 15% (Fig. 4B). Exogenous application of silicon at both doses improved significantly ($P < 0.01$) the total acidity of tomato under water stress conditions (Fig 5C). Moreover, there was a 20% increase in TSS when using 200 mg L⁻¹ Si (Fig. 3B).

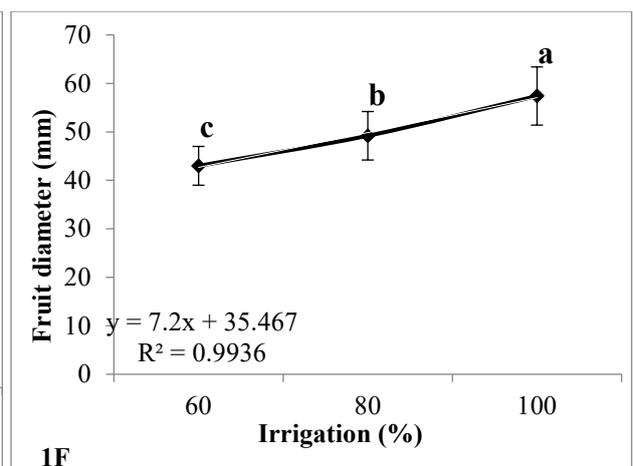
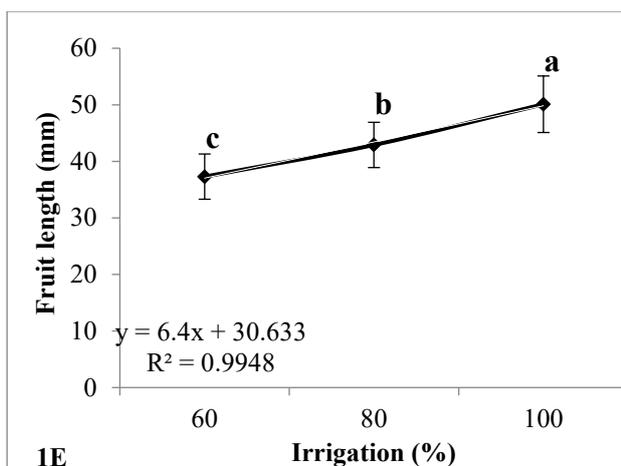
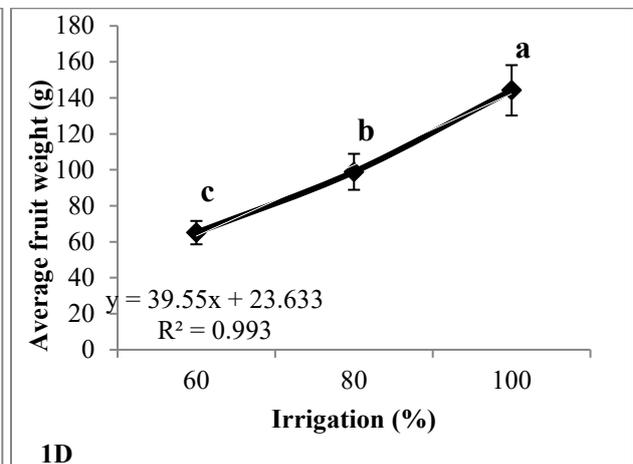
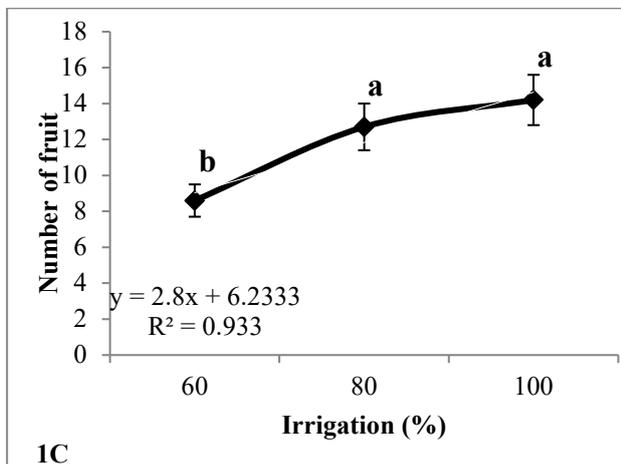
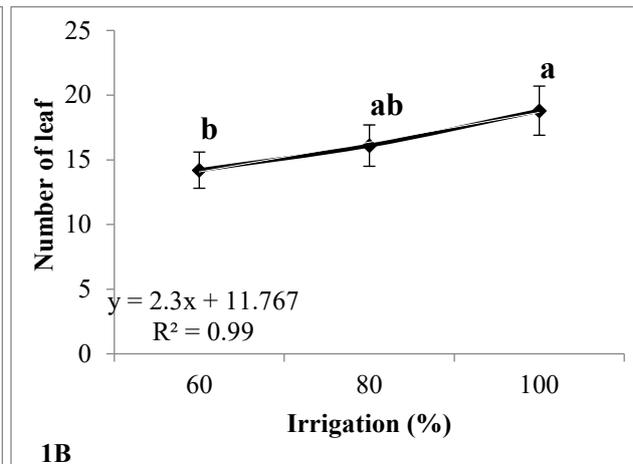
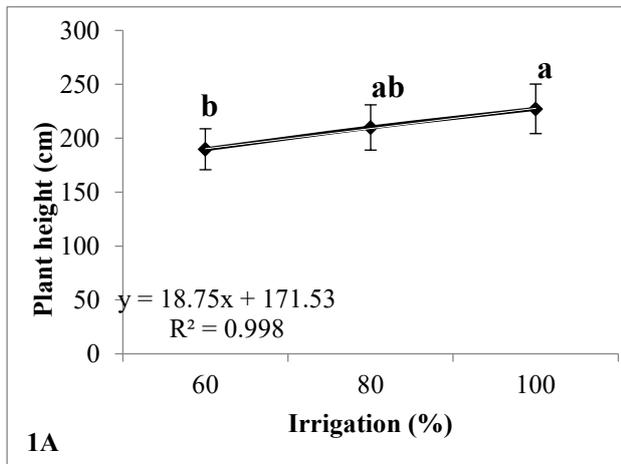


Fig. 1. The effects of irrigation levels on the plant height (1A), the number of leaf (1B), the number of fruit (1C), the average fruit weight (1D), the fruit length (1E) and the fruit diameter (1F)

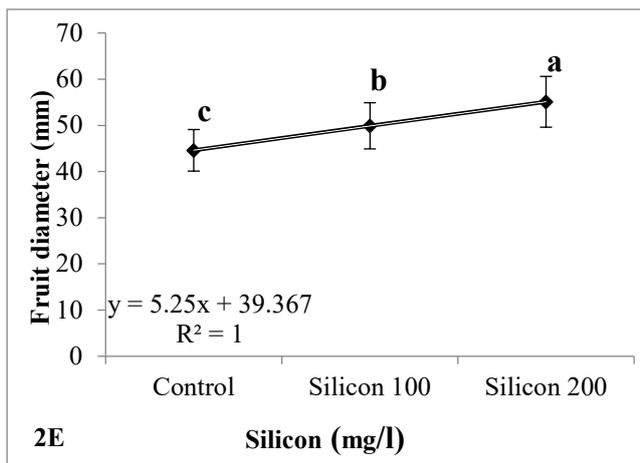
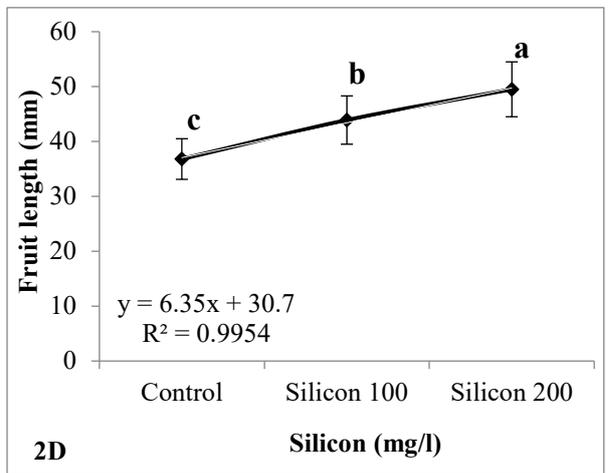
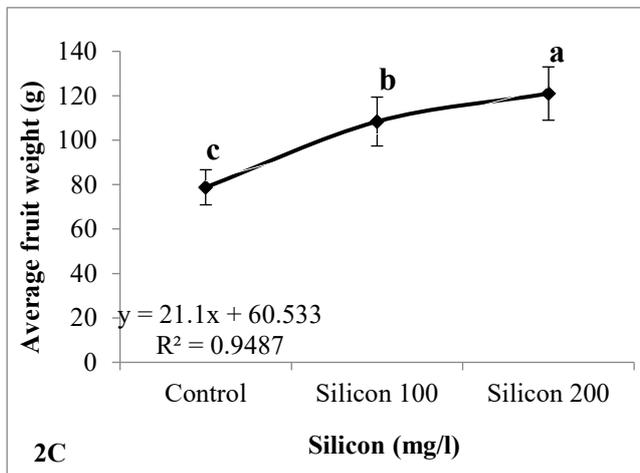
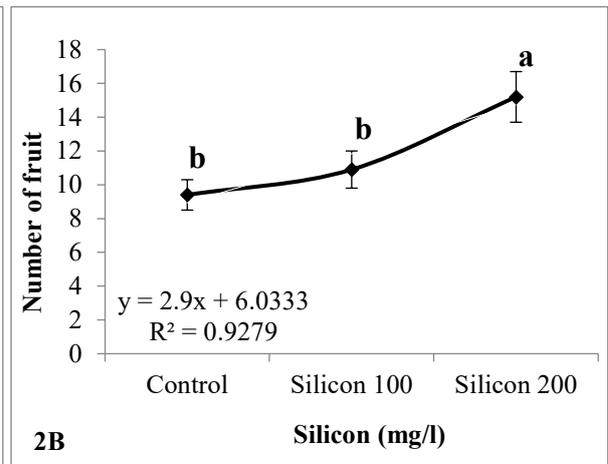
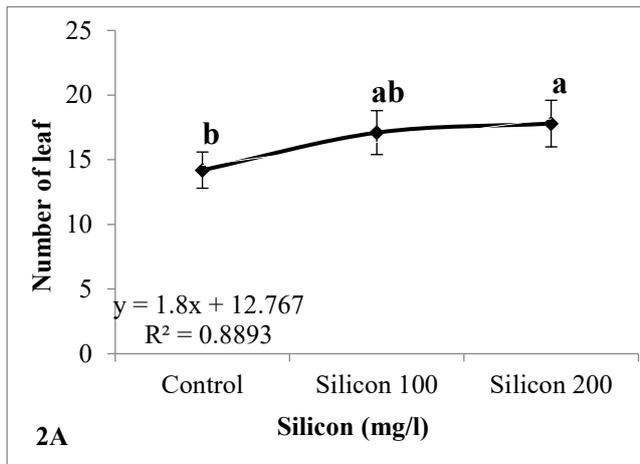


Fig. 2. The effects of silicon levels on the number of leaf (2A), the number of fruit (2C), the average fruit weight (2D), the fruit length (2E), and the fruit diameter (2F)

Similarly, the maximum fruit firmness (3A) and vitamin C (3C) were shown in the group that received the Si fertilizer at both doses as compared with the control group.

Effect of water deficit and silicon on antioxidant capacity, total flavonoids, and antioxidant enzyme activities

Considering mean values, total flavonoids were significantly ($P < 0.01$) affected by the irrigation and Si levels individually and their combination together (Tab. 1). The highest flavonoids content was found in the plant treated with 200 mg L⁻¹ Si under severe water stress (60%) compared to the group received the full water requirement of the tomato (Fig. 5A). The foliar application of Si at 200 mg L⁻¹ significantly increased total flavonoids from 0.1 to 0.4 gL⁻¹ in the group ex-

posed to severe stress (Fig. 5B). Similarly, the mild and severe water stress conditions increased the antioxidant capacity by 15% and 40%. The activities of CAT (Fig. 5D) and POX (Fig. 4D) were significantly increased due to the severe water stress, by about 75% and 60%. In comparison to the control group, Si application at 100 mg L⁻¹ dose caused a significant ($P < 0.01$) increase in POX activity by about 12%. The results indicated that the application of Si at the doses of 100 and 200 mg L⁻¹ could increase remarkably ($P < 0.01$) the CAT (Fig. 5D) activity.

Effect of water deficit and silicon on fruit nitrogen, potassium, and Si contents

Our results demonstrated that the irrigation treatment has significantly ($P < 0.01$) influenced the nitrogen, potassium, and Si concentrations (Tab 1). The

Table 1. ANOVA table of the effect of irrigation and Si treatments on the tested parameters

Dependent variable	Independent variable				
	irrigation (I)	silicon (Si)	I × Si	error	CV%
Plant length	3179.7**	1231.8 ^{ns}	209.5 ^{ns}	499.9	10.7
Number of leaves	48.36*	32.86*	6.56 ^{ns}	8.69	18.0
Number of clusters	15.36**	6.69 ^{ns}	0.72 ^{ns}	1.92	19.3
Number of flowers in clusters	10.03**	4.75*	0.53 ^{ns}	1.25	15.1
Number of fruits	77.6**	82.6**	0.4 ^{ns}	2.5	13.3
Average fruit weight	14181.9**	4230.7**	248.2 ^{ns}	119.1	10.6
Fruit length	372.1**	364.5**	18.3 ^{ns}	12.8	8.2
Fruit diameter	468.4**	247.8**	4.9 ^{ns}	11.4	6.8
Yield	2704.3**	874.3**	108.3**	19.7	2.7
Fruit firmness	0.567 ^{ns}	0.880*	0.064 ^{ns}	0.213	22.0
Total soluble solid	5.57**	2.71**	0.33 ^{ns}	0.41	12.4
Total acid	1.103**	0.192**	0.058**	0.012	11.8
Vitamin C	71.3**	128.0**	5.9 ^{ns}	13.6	12.9
Total flavonoid	0.0409**	0.0396**	0.0090**	0.0016	20.7
Antioxidant	1.183*	0.261 ^{ns}	0.017 ^{ns}	0.214	19.9
Catalase	0.0116**	0.0152**	0.0004**	0.0001	10.1
Peroxidase	0.6169**	0.0199**	0.0005 ^{ns}	0.0007	3.9
Potassium (K)	0.1016**	0.0369 ^{ns}	0.0019 ^{ns}	0.015	13.0
Nitrogen (N)	0.143**	0.019 ^{ns}	0.001 ^{ns}	0.009	6.9
Silicium	3.099**	1.607**	0.021 ^{ns}	0.240	18.2

^{ns}, * and ** are non-significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively

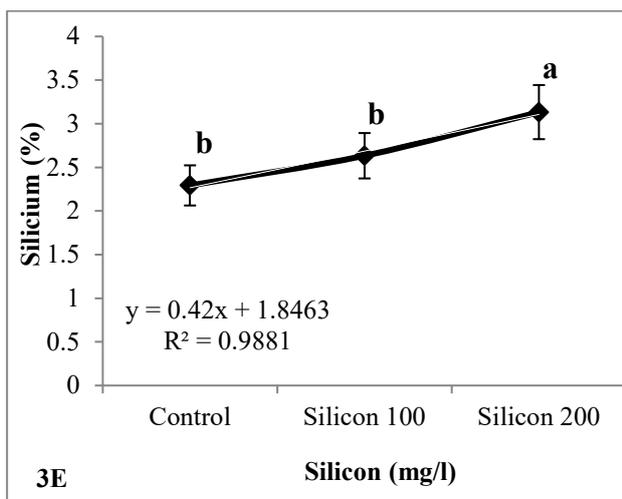
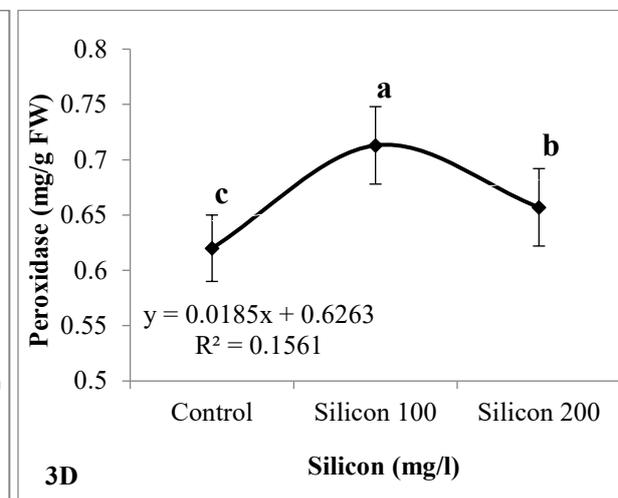
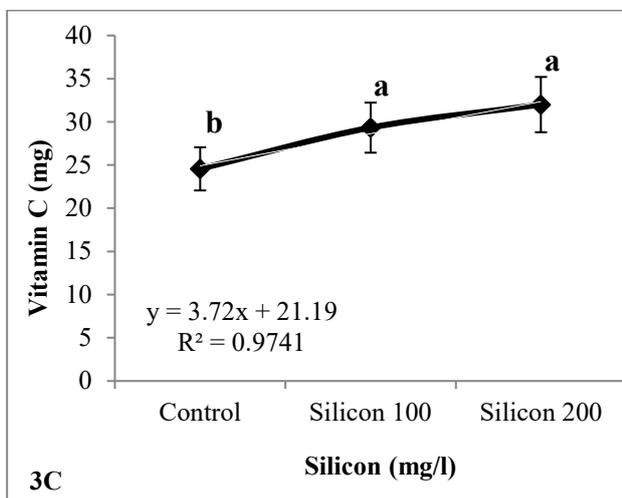
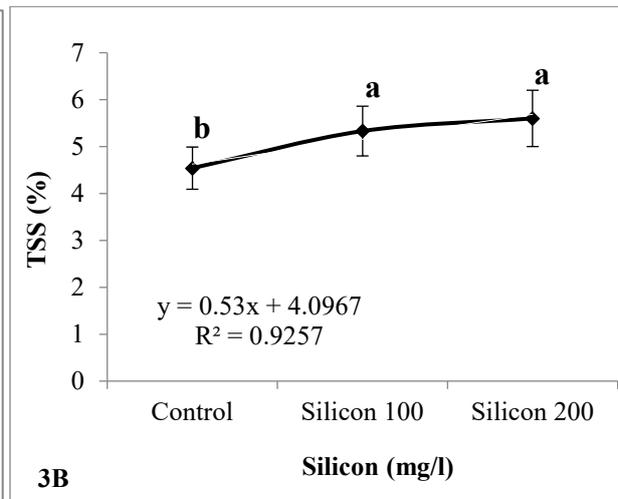
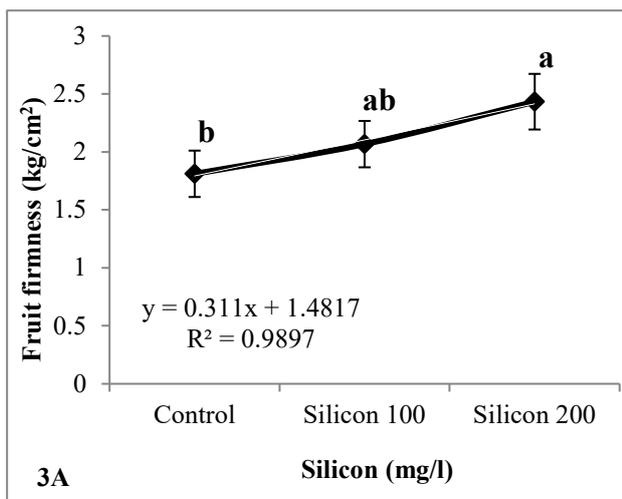
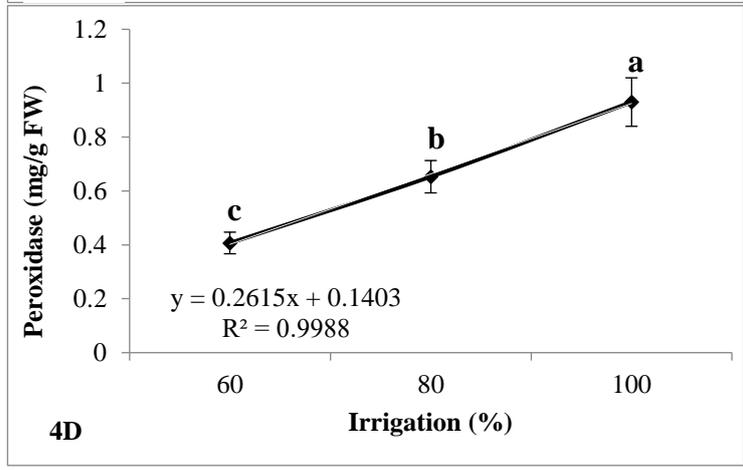
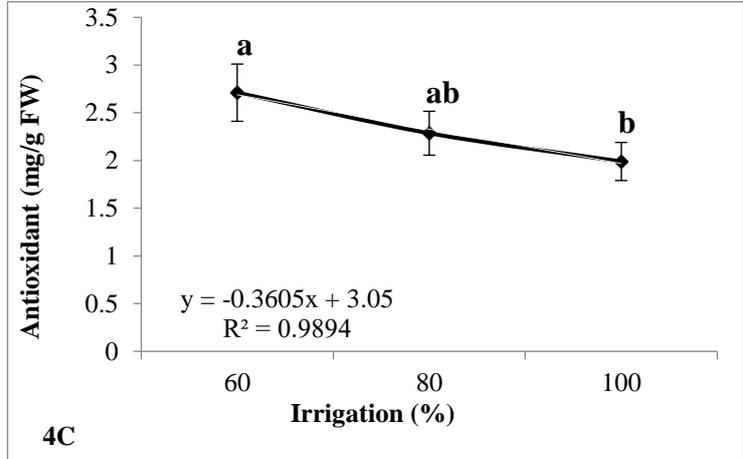
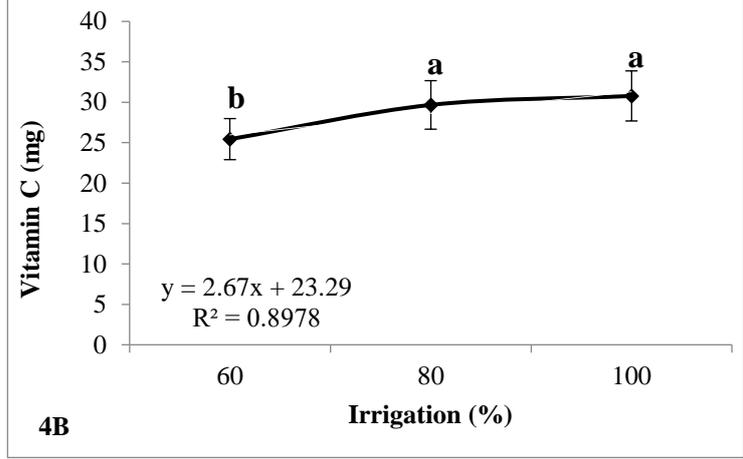
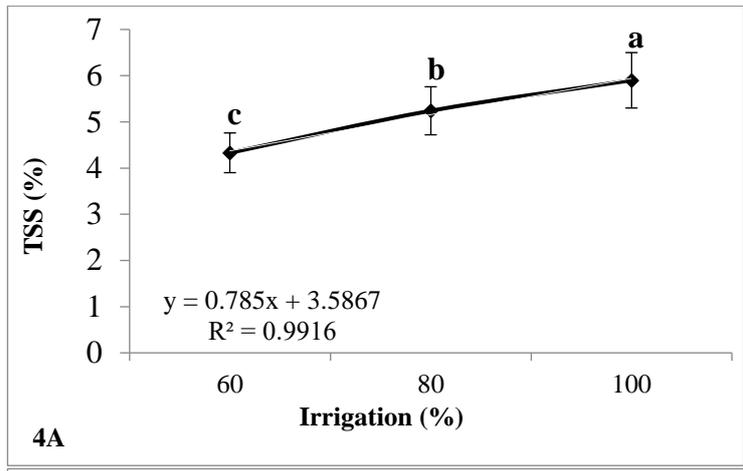


Fig. 3. The effect of Si levels on the fruit firmness (3A), TSS (3B), vitamin C (3C), peroxidase (3D), and silicium (3E)



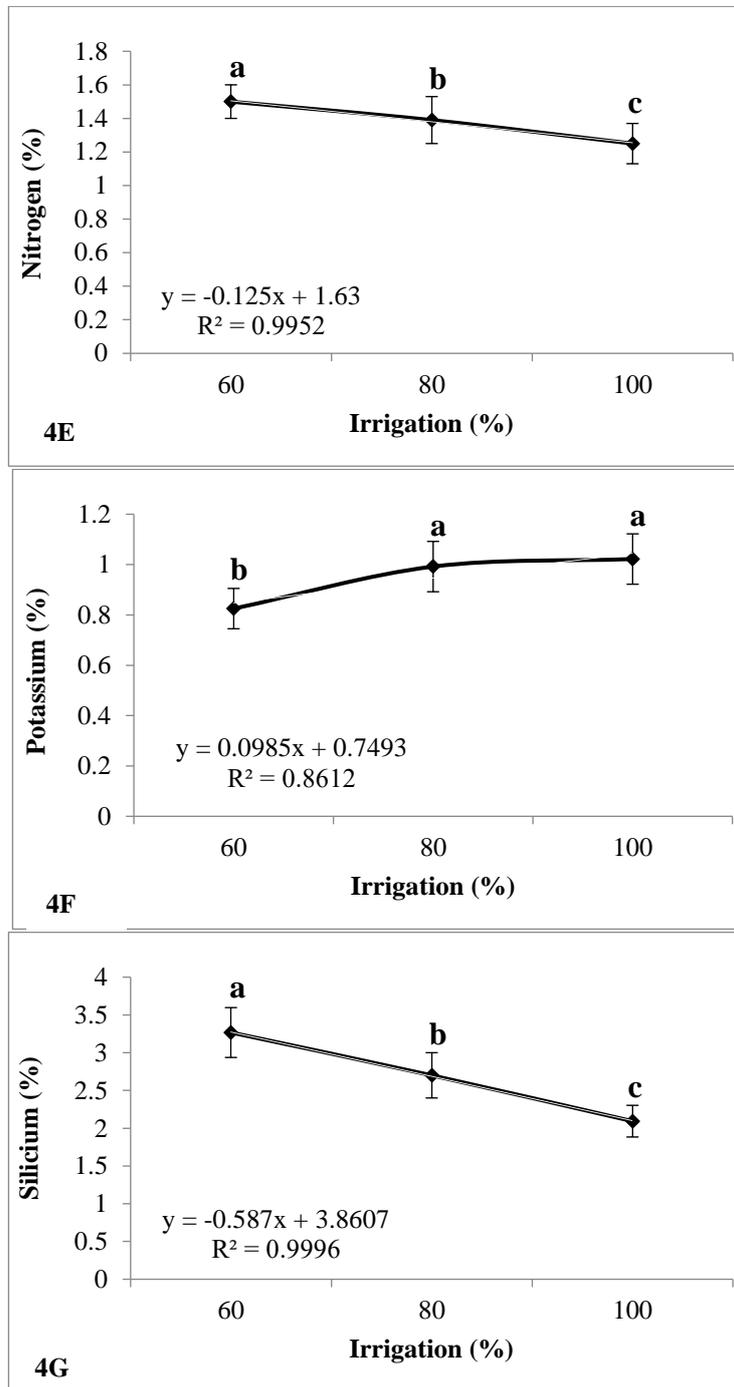


Fig. 4. The effect of irrigation levels on TSS (4A), vitamin C (4B), antioxidant capacity (4C), peroxidase (4D), nitrogen (4E), potassium (4F), and silicon (4G)

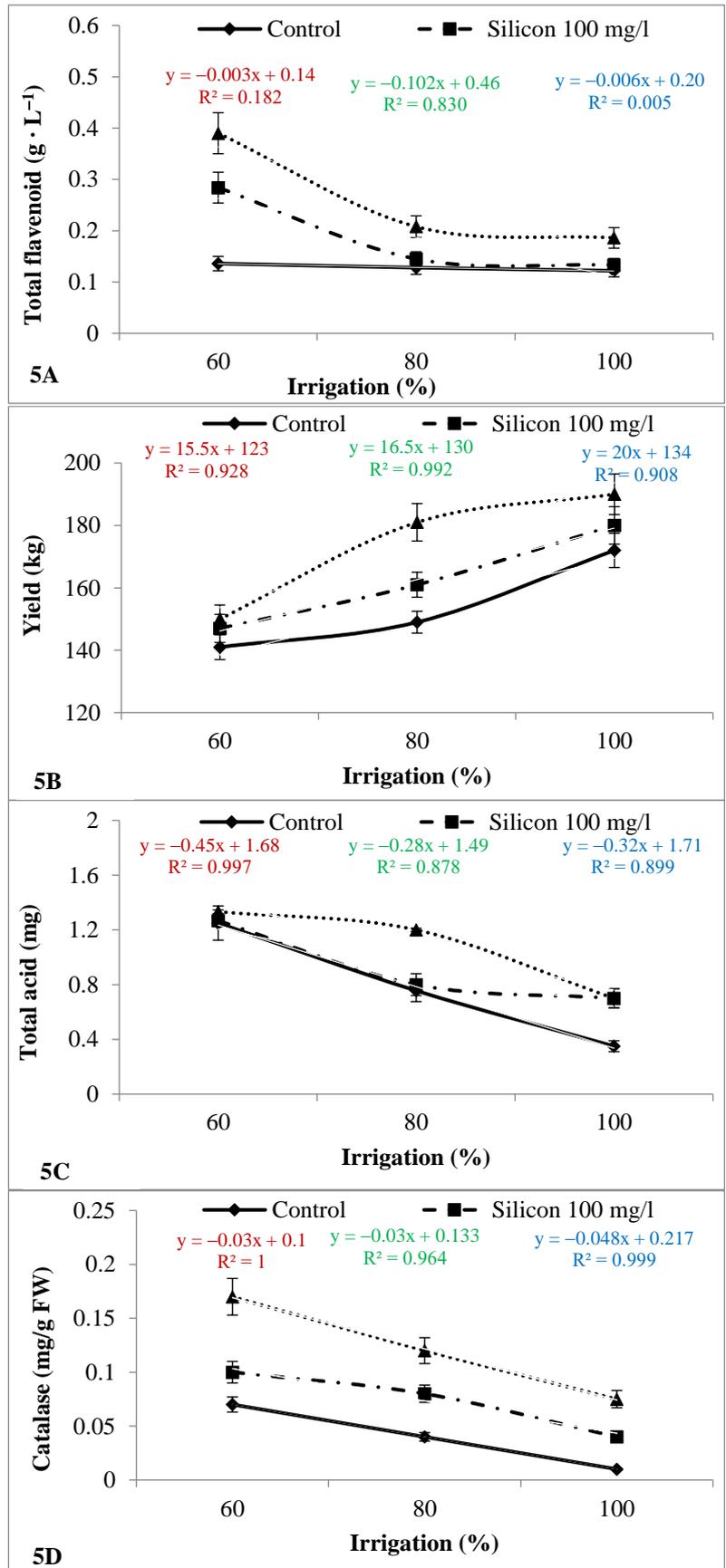


Fig. 5. The effect of interaction of irrigation levels and Si on total flavenoids (5A), yield (5B), total acid (5C), and catalase

contrary to K value, concentrations of Si (Fig. 4G) and N (Fig. 4E) increased significantly under severe water stress, by about 50% and 25%, respectively. Similarly, the foliar application of Si at both doses (Fig. 3E) caused a notable increase in Si content when compared to the control group.

DISCUSSION

Water scarcity has become one of the world's most serious environmental issues, especially in arid and semi-arid regions like Iran which could limit agricultural products [Faramarzi et al. 2010]. The growth and yield of many crops such as *Zea mays* L. [De Araujo Rufino et al. 2018] and *Eragrostis tef* (Zucc.) [Araya et al. 2011] were severely influenced by water stress. The current study found a loss of approximately 25% in tomato yield due to water stress (Fig. 5B). As recently reported by Golmohammadi et al. [2020] and Seymen et al. [2020], water deficiency also showed a negative effect on the fruit-set and resulted in an extreme loss in the yield of olive and pumpkin. However, Si, as a mineral, is believed to regulate different physio-biochemical processes in plants exposed to environmental stress including photosynthesis, stomatal regulation, and ion uptake. Thus, Si has potential roles in activating plant growth and productivity [Mauad et al. 2016, Ali and Hassan 2017]. In the current study, the foliar spray of Si (200 mg L⁻¹) was able to increase tomato yield by almost 25% under mild stress (Fig. 5A). These results are in line with reports for *Triticum aestivum* L. [Maghsoudi et al. 2019] and *Zea mays* [Sirisuntornlak et al. 2019]. Moreover, the results of the current study demonstrated that mild and severe water stress remarkably reduced the growth parameters of tomatoes including the leaf numbers and the plant height (Tab. 1). It is well documented that water stress firstly decreased the turgor which resulted in a reduction in cell development of leaves and stems and consequently reduced the growth [Hassan et al. 2013]. In this sense, the plant morphology was altered due to water stress including the plant height and shoot growth [Dehghanipoodeh et al. 2018]. In accordance with our results, Al-Huqail et al. [2020] found that basil plant growth (the number of leaves and plant height) was significantly reduced under water stress [Al-Huqail et al. 2020]. Furthermore, water

stress significantly decreased the fruit number as well as fruit quality (fruit size and fruit weight) (Tab. 1) which is in agreement with a previous study on cherry tomatoes exposed to deficit irrigation [Cantore et al. 2016]. The reduction in fruit number and weight contributes to the reduction in yield which was observed in the current study like previous studies [Chen et al. 2013, Hao et al. 2013]. In general, a reduction in fruit weight is dependent upon the duration and intensity of the stress and a reduction of fruit is mainly due to the abortion of the flowers or the early drop of small fruits [Cantore et al. 2016]. The foliar application of Si (200 mg L⁻¹) had significant ($P < 0.01$) effects on the morphological traits of tomato e.g., fruit weight, fruit size, and fruit number. These results are in accordance with previous studies which reported that the application of Si could enhance the growth parameters, yield, and quality of the crop [Hellal et al. 2012, Van Bockhaven et al. 2013, Ullah et al. 2016]. The improvement in TSS and titratable acidity under water deficiency was observed in the current experiment which is consistent with other results [Patanè and Cosentino 2010, Patanè et al. 2011]. Generally, increasing the amount of TSS in plants under water stress is a trick that plants use to regulate osmosis and use to withstand stress [Giné-Bordonaba and Terry 2016].

In comparison to the control group, the application of Si at both doses (100 and 200 mg L⁻¹) remarkably enhanced the fruit firmness ($P < 0.05$) and TSS ($P < 0.01$) which were in accordance with the findings of González-MoscOSO et al. [2019], who observed that the silicon nanoparticles (SiO₂ NPs) had positive effects on two parameters [González-MoscOSO et al. 2019]. In a study by Zahedi et al. [2020], the fruit firmness of strawberries decreased due to the drought stress, however, the use of Si could improve its fruit firmness [Zahedi et al. 2020]. Similarly, a remarkable evaluation of TA was found in the group exposed to severe water stress in grapes [Mirás-Avalos and Intrigliolo 2017], tomato [Sobeih et al. 2004], and strawberries [Bordonaba and Terry 2010]. In addition, the foliar application of Si enhanced the value of vitamin C (Fig. 3C) which is in line with the study of Shalaby et al. [2017]. Vitamin C, as a water-soluble antioxidant, is an efficient scavenger of free radicals to protect plant cells against oxidative damage [Smirnoff and Wheeler 2000, Beltagi 2008]. The previous inves-

tigation confirmed that vitamin C could regulate the antioxidant defense metabolism in *Hordeum vulgare* [Agami 2014] and *Brassica napus* [Shafiq et al. 2014], under salinity and water stresses, respectively.

Due to abiotic stresses like water and salinity, the balance between ROS generation and ROS scavenging is disturbed leading to the degradation of biomolecules namely, proteins, lipids, and DNA [Das and Roychoudhury 2014, Attaran et al. 2018, Fatemi et al. 2019, Fatemi et al. 2020]. To cope with these undesirable conditions, plants have enzymatic (SOD, CAT, GPX, and GST) as well as non-enzymatic (total flavonoids and phenols) strategies [Pandey et al. 2017, Sofy et al. 2020]. The present research depicted that the content of total flavonoids in tomatoes was influenced by water stress, but the foliar application of 200 mg L⁻¹ Si improved this parameter. These results are in accordance with the results of Ahmad et al. [2021], who demonstrated a considerable increase in antioxidant contents in camelina plants treated with 6 mM Si under drought stress. Studies on tomatoes [Klunklin and Savage 2017] and soybean [Mohamed and Akladios 2014] demonstrated that the water stress caused a significant increase in the levels of antioxidant activities estimated by DPPH and ABTS tests which are agreed with the current consequences. In another study, the accumulation of flavonoids, as a secondary metabolite, in *Arabidopsis thaliana* L. was increased due to inducing resistance against the water stress [Shojaie et al. 2016] which matches our findings (Tab. 1). The Si application could increase the levels of phenolic and flavonoid content of rice when subjected to the water stress [Emam et al. 2014]. Water-limited conditions markedly promoted the activities of key antioxidant enzymes namely CAT and POX in tomatoes [Murshed et al. 2013]. These results are in agreement with our previous study on the tomato Rio Grande cultivar, the antioxidant enzyme activities (SOD and CAT) significantly increased in response to water stress [Barzegar et al. 2019]. The influence of Si on the activities of antioxidant enzymes differs according to the plant species and time [Zhu and Gong 2014]. The administration of Si can improve the ability of ROS scavenging by regulation of antioxidants enzyme activity [Torabo et al. 2015, Tripathi et al. 2015]. It has been confirmed by several studies on tomato [Shi et al. 2016], *Spinacia oleracea* L. [Gunes et al. 2007], and maize [Li et al. 2007].

An increase in mineral nutrients (e.g., K⁺ and Mg²⁺) is believed to be another critical mechanism for plants to resist stress. In the present investigation, accumulation of N and Si took place in the subjected water-limited conditions, and foliar-applied Si caused a further enhancement in Si levels. Changing the nutritional elements during the water shortage may be due to reducing transpiration flow and uptake of the essential nutrient [McWilliams 2003]. In a study, the silica nanoparticles enhanced the uptake of nitrogen, potassium, and silicon in all plant tissues [Alsaedi et al. 2019].

CONCLUSIONS

The present results revealed that severe and mild water restriction has negative effects on growth parameters, tomato yield, and fruit quality. However, the application of 200 mg L⁻¹ Si could improve plant growth and increase tomato yield by up to 25%. In addition, a significant increase of total flavonoids and TA was detected with increasing water-deficit stress. The highest value of CAT and POX activities and antioxidant capacity were obtained in tomatoes under water stress. The foliar application of Si at both 100 and 200 mg L⁻¹ markedly improved antioxidant activities and total flavonoids which is a key role in promoting plant resistance against water stress. Therefore, tomato is a good candidate crop to benefit from Si for managing water stress.

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