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Shading net and foliar applications of Si and K alleviates heat stress and improves pomegranate fruit quality

Samira Moradi[1], Zabihollah Zamani[1]✉, Reza Fatahi[1], Mahmoud Koushesh-Saba[2], Daniela Pampinella[3], Vito Armando Laudicina[2,3], Paolo Inglese[3] & Giorgia Liguori[3]

High temperature and light intensity are limiting factors for reducing quality during pomegranate fruit growth and development. In this research, mitigation of the heat stress detrimental effects was investigated on pomegranate through the use of a shading net and foliar application of silicon and potassium nutrient elements. Treatments included net shading (0 and 50%) and foliar spraying of trees during the growing season with K (1% and 2%) and Si (0.05%, 0.1%, and 0.15%). The foliar sprays were applied three times, first after fruit set and then twice after with one-month intervals. Shading net increased leaf chlorophyll content, net photosynthetic rate, transpiration, substomatal CO_2, relative water content, and antioxidant enzymes activity. Shading net guards membrane integrity, being obvious through the reduction of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents in pomegranate leaves. Shading net significantly reduced the percentage of fruit sunburn to 12% and cracking to 11% on compared to the control (41% and 27%). Si and K applications were also effective on the physiology of pomegranate trees in response to heat stress. Si applications increased superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), chlorophyll content, and relative water content, while reduced MDA and H_2O_2 contents. Shading net and Si spraying positively affected the uptake of macronutrients while having no significant effect on leaf micronutrients. High temperatures adversely affected the recorded responses of the pomegranate trees and, the shading net effectively reduced heat-induced disorders. In addition, application of Si and K ameliorated the negative effects of heat stress.

Global temperature rise leading to climate change has become a major problem in the world[1]. These have led to excessive weather phenomena, especially in tropical and subtropical fruit-growing areas, and caused considerable economic loss in the horticulture industry[2]. High light intensity and temperature in the tree canopy, low water availability and high salinity are the main factors prevailing in pomegranate growing areas. Commercial pomegranate cultivation worldwide occurs in subtropical, semi-arid climates characterized by high solar radiation and high summer temperatures; therefore, heat stress occurs in these regions during the pomegranate growing season. Iran is one of the largest pomegranate producers in the world[3]. Most of Iran's pomegranate production takes place in desert areas, and in these areas hot summers cause severe stress to the plants and their production. Limited water resources and salinity are also the main reasons for causing stress to plants in these areas. It is reported that abiotic stresses, especially higher temperatures, can negatively affect physiological and biochemical processes in plants[4]. In addition, high light intensity leads to the development of physiological disorders such as sunburn of fruit, causing great economic loss to producers[5]. In these areas, physiological disorders on pomegranate fruit such as fruit sunburn and cracking are very common causing huge waste in production chain. In addition to high temperature and sun exposure, fruit cracking is also strongly influenced by water availability. Sudden changes in water status such as rainfall events following prolonged periods of water stress can lead to an abrupt increase in fruit turgor pressure, promoting peel rupture and cracking. This phenomenon has been documented in several fruit species, including pomegranate[6], and is particularly relevant even in hot and arid climates where intermittent rainfall can occur.

[1]Department of Horticulture Science, Faculty of Agriculture, University of Tehran, Karaj, Iran. [2]Department of Horticulture Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Kurdistan, Iran. [3]Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy. ✉email: zzamani@ut.ac.ir

Several strategies have been developed to reduce the severity of abiotic stressors to plants. Among the most important ones are the use of shade nets to protect leaves and fruit from direct sunlight[7], and foliar application of nutrients to improve plant health and vigor[8]. Shading nets provide an appropriate microclimate for physiological processes leading to reduce physiological disorders and increase antioxidant traits, and led to higher fruitquality[9,10]. Shading nets are an agrotechnical advancement leading to changes in light intensity and quality for the commodity, resulting in improvement in thermal conditions and protecting agricultural crops from excessive solar radiation[11]. Various environmental elements have been shown to be affected by shading nets such as light intensity and quality[4], canopy and soil temperature[12] and relative humidity[13]. Shading nets reduce the daily maximum temperature in Mediterranean climates[13]. Shading nets can effectively reduce canopy temperature and evapotranspiration rates during high ambient temperatures, thus protecting photosynthetic activity[14].

Excessive and inappropriate use of chemical fertilizers has remarkable negative impacts on environmental sustainability, and excessive amounts of applied nutrients may be lost from agricultural lands[15], resulting in unpredictable damages to the biosphere. There is an increasing interest in returning to nature or creating sustainable environmental conditions by promoting the least use of fertilizers in agricultural production, being referred to as green agriculture. Fortunately, besides their roots, the aboveground parts of plants, especially the leaves, can absorb nutrients[16], so applying minerals on the leaves with the least chemicals is an effective method for supplying their nutrient needs. Mineral nutrients provide the basic needs of plants for growth and development, as well as protecting them from various stresses by strengthening the plant's structure and enhancing their defense mechanisms[17].

Potassium (K) is one of the most important macronutrients for plant growth and development, playing a key role in water regulation, enzyme activation, and stress tolerance. The significant effects of K on protein synthesis, enzyme activation, and photosynthesis have been confirmed[18]. Potassium reduces the effects of various stresses on plants, including drought, cold, and high light intensity[19]. A combined tolerance to both high temperature and drought in plants, induced by K has been reported[25].

There are many reports on the positive role of Silicon (Si) on plant growth under abiotic stress conditions such as salinity, metal toxicity, drought, radiation damage, high temperature, and frost . Indeed, Si has a significant impact on stress tolerance mechanisms leading to improvements in growth characteristics, photosynthetic rate, and nutrient uptake, as well as activation of the antioxidant defense system in plant tissue[29], mitigating plant stress, especially at high temperatures[26]. The effects of Si vary depending on the plant species, but in general, it increases immediate water use efficiency and photosynthesis[22,27,28]. Foliar application of Si reduced lipid peroxidation, electrolyte loss, and superoxide and hydrogen peroxide levels as the major reactive oxygen species[27].

However, not much is known about the effects of silicon on pomegranate trees under different stress conditions. Therefore, the objective of this research was to study the effect of K and Si supplied by foliar application in improving physiological and photosynthetic parameters and alleviating important physiological disorders in pomegranate trees grown at harsh environmental conditions and under a shading net.

Materials and methods

Plant material and treatments

This study was conducted in Qom province (34° 38' 23.9964" N and 50° 52' 35.0004" E), which is located in central Iran and characterized by a hot, arid climate (Fig. 1). The experiment was conducted in the 2020 and 2021 growing seasons on 11-year-old trees of pomegranate (*Punica granatum* L.) cv. Ghojagh with a tree spacing of 4 × 4 m N-S oriented. Pomegranate 'Ghojagh' has large fruit with red skin, red arils, pleasant, sweet-sour taste, and good shelf life with commercial quality. Treatments included net shading (0 and 50%) and foliar application of trees with K (as potassium sulphate) and Si (as sodium silicate) during the growing season.

In this experiment, pomegranate trees under shading net (green 50%) and non-shaded trees (in full sun, as control) were used (Fig. 2). The green 50% shading nets were applied to the trees as described in Moradi et al.[29].

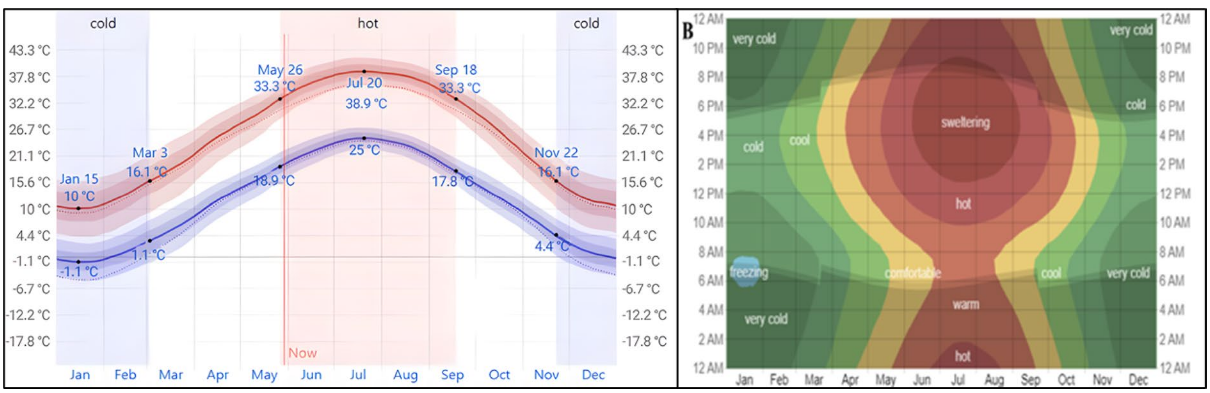

Fig. 1. Average highest and lowest temperature and average hourly temperature in Qom province (https://weatherspark.com/y/104978/Average-Weather-in-Qom-Iran-Year-Round).

Fig. 2. Installation of shading net on pomegranate trees.

Sodium silicate at three concentrations (0.05, 0.1, and 0.15%) and potassium sulphate at two concentrations (1% and 2%) were used for nutrient applications. Foliar applications were performed at three stages, once after fruit set and twice later at one-month intervals (July 2, August 2, and September 2 respectively). During the study period, trees were irrigated using a drip irrigation system to ensure uniform water distribution and minimize fluctuations in water availability. This method is particularly relevant under arid conditions, as inconsistent water supply can exacerbate physiological disorders such as sunburn and fruit cracking[5]. Irrigation was applied approximately every 10 days from May to September, depending on weather conditions. No fertilization was applied during the study period. Weed control was performed manually, and no pesticides were used.

Healthy and fully developed leaves were collected at three time points during the experiment, (a week after each spraying, and transferred to the laboratory in ice-cooled boxes in sealed plastic bags. The fresh leaf samples were used to measure traits such as relative water content, and an additional leaf sample was frozen for other measurements. The frozen leaves were used to measure chlorophyll content, malondialdehyde, hydrogen peroxide, total protein content, and antioxidant enzyme activity. The experiment was performed in a split-plot arrangement with four replicates (blocks) and four trees per replication. The main plots were net shading (0 and 50%) and the sub-plots were nutrient applications (sodium silicate and potassium sulfate).

Physiological traits

Photosynthetic parameters

Trees physiological parameters, including photosynthetic activity as well as water use efficiency, mesophyll conductance (MC), leaf surface temperature, canopy temperature, and light intensity were measured. Measurements were carried out one week after the second stage of foliar spraying on the fully developed leaves of the southwest side of the 48 trees between 9:00 and 10:00 am, when the temperature was between 30 °C and 33 °C. This experiment included 12 treatments (6 treatments in open field and 6 treatments under shading net), including one control, three silicon concentrations, and two potassium concentrations. For each treatment, four trees were randomly selected (totaling 48 trees). On each tree, three fully expanded leaves exposed to sunlight were measured, resulting in 12 replicates per treatment for physiological parameters.

Net photosynthesis (μmol CO_2 m^{-2} s^{-1}), substomatal CO_2 (mmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs) (mmol m^{-2} s^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), and leaf surface temperature, were measured using a photosynthesis analyzer instrument (LCI- ABC-UK). Canopy temperature (°C) was recorded with an infrared thermometer (Testo 830-T1, Germany) at 50 cm from the leaf surface and with a measurement field diameter of 66 mm. A SunScan analyzer (type SS1, England) was used to measure photosynthetically active radiation (PAR). Water use efficiency (WUE)[29] and MC[32] were calculated using the following equations:

$$\mathrm{WUE} = \frac{\text{photosynthetic rate}}{\text{transpiration rate}}$$

$$\mathrm{MC} = \frac{\text{photosynthetic rate}}{\text{substomatal } \mathrm{CO_2}}$$

Relative water content (RWC)

RWC measurements were performed on fully developed leaves. Each leaf was divided into several parts with a scalpel and then weighed (fresh weight, FW). To determine turgid weight (TW), leaves were immersed in a Petri-dish containing distilled water and kept under dark and normal temperature conditions in the laboratory. During the water absorption period, leaf samples were weighed at regular intervals after carefully wiping the water from the leaf's surface until a stable weight was reached. To determine the dry weight (DW), leaf samples were placed for 48 h in an oven preheated to 80 °C [33]. RWC was calculated according to the following equation:

$$\mathrm{RWC}\ (\%) = \left[{}^{(\mathrm{FW - DW})}/_{(\mathrm{TW - DW})} \right]$$

Chlorophyll content

Total chlorophylls were extracted with 80% acetone and measured according to Lichtenthaler & Buschmann[32]. In summary, 0.1 g of fresh leaf tissue was homogenized in 5 mL of 80% acetone. The homogenate was centrifuged at 5,000 rpm for 10 min to separate the supernatant. Absorbance was measured at 646 and 663 nm using a spectrophotometer. Four replicates were analyzed per treatment.

Assay of enzymes activity

A sample of frozen leaves (0.5 g) was homogenized with 1 ml of extraction medium containing 100 mM phosphate buffer (pH = 7), 2 mM EDTA, and 1% (w/v) polyvinylpyrrolidone (PVP). After centrifugation of the homogenate at 10,000 g for 20 min, the supernatant was used for enzyme assays. All the above steps were performed at 4 °C. Protein content was evaluated as described by Bradford[34] and using bovine serum albumin concentrations as standard. Peroxidase (POX) activity was determined by the rate of guaiacol oxidation in the presence of H_2O_2 at 470 nm for 1 min, as described previously[33]. Superoxide dismutase activity (SOD) was determined according to Giannopolitis & Ries[35] with some modifications, by measuring the absorbance of the mixture at 560 nm after exposure to light for 30 min. CAT activity was determined by measuring the rate of disappearance of H_2O_2 according to the method of Chance and Maehly[36]. The reduction of H_2O_2 was followed as a decrease in absorbance at 240 nm.

Determination of MDA content

A sample (0.1 g) of frozen leaf tissue was homogenized in 5 mL of 10% trichloroacetic acid (TCA) and centrifuged for 10 min at 10,000 g and 4 °C to measure malondialdehyde content. MDA content was then determined according to Dhindsa et al.[37]. The extract was mixed with an equal volume of a 0.5% thiobarbituric acid including 5% TCA, after that heated at 100 °C for 15 min, then the absorbance was measured after centrifugation at 532 nm and 600 nm. MDA content was expressed as nmol g^{-1} fresh leaf weight.

Determination of H_2O_2 content

A sample of frozen leaf tissue from each replicate (0.15 g) was homogenized in 5 ml of 0.1% TCA and centrifuged at 4 °C for 10 min at 10,000 g. An aliquot of the supernatant (250 µL) was then mixed with 250 µL of phosphate buffer (0.1 M) and 500 µL of 1 mM potassium iodide (KI), shaken, and incubated at room temperature for 2 h, at which time color development was virtually complete. The absorbance at 390 nm was read after centrifugation for the removal of all flocculated materials. H_2O_2 content was determined in µmol g^{-1} fresh weight[38].

Evaluating fruit sunburn and cracking severity

In our study, sunburn and fruit cracking were assessed on the southwest side of the tree canopy. This orientation was selected because, in our geographical location, the southwest side receives the most intense sunlight exposure during the hottest part of the day—typically afternoon. This prolonged and direct solar radiation increases the risk of heat-related physiological disorders such as sunburn and cracking. Therefore, evaluating this side provides a consistent and sensitive basis for detecting treatment effects. This approach may differ from methods used in species or climates where sunburn is not a major concern and where fruit cracking is assessed more uniformly around the canopy or on marked branches at earlier developmental stages.

At harvest time, fruit on the southwest side of the trees were counted, and each fruit was visually assessed for the presence of sunburn or cracking damage, and data were calculated as a percentage relative to the total number of fruits on the southwest side of the tree as sample per replicate according to the following equations.

$$\text{Sunburn fruit } (\%) = \frac{\text{Number of sunburn fruit}}{\text{Total number of fruits on the southwest side of the tree}} \times 100$$

$$\text{Cracking fruit } (\%) = \frac{\text{Number of cracked fruit}}{\text{Total number of fruits on the southwest side of the tree}} \times 100.$$

Mineral nutrients analysis

Leaf samples (20 fully developed leaves from new growth) from experimental plants were dried at 60 °C and analyzed for mineral nutrient concentrations. The dried leaf powder (0.5 g) was ashed in a furnace at 550 ± 25 °C for 5 h. The resulting white ash was then dissolved in 15 mL of 2% HNO_3 for the determination of macro- and microelements[39]. Suitable dilutions were subsequently made for the determination of various elements. The contents of Ca, Mg, K, Si, Fe, Na, Cu, Mn, and Zn were determined by a microwave plasma-atomic emission spectrometer (MP-AES) system (Agilent 4210 MP-AES; Milan, Italy).

For the determination of total nitrogen 0.2 g of leaf sample was weighed into the digestion tube, 5 mL of concentrated sulfuric acid and a small amount of copper (II) oxide catalyst were added. The sample was mineralized at 360 °C for 5 h, with DKL20 digestion unit (VELP® Scientifica). The mixture was cooled to room temperature. Total nitrogen (TN) was determined using the Kjeldahl distillation apparatus UDK (VELP® Scientifica) and an automatic titrator metrohm (916 Ti-Touch)[40].

Total phosphorus (P) was determined by the colorimetric method on the mineralized sample. Briefly, the method involves the addition of 0.5 mL mineralized sample, 4.5 mL H_2O, 0.25 mL H_2SO_4 (5 M) and 1 mL reagent solution prepared with ascorbic acid, ammonium molybdate, and antimony potassium tartrate, which reacts with phosphate ions to form a blue-molybdate complex. The intensity of the color is proportional to the phosphate concentration in the sample. Spectrophotometer (UVmini-1240, Shimadzu Italia srl, Milan Italy) was used to measure the absorbance of the colored complex at 882 nm. The absorbance value was then compared to a calibration curve generated using standard phosphate concentrations (2.5, 5, 10, 15 and 20 mg L^{-1})[43].

Statistical analysis

First, a test was performed to compare between two years, considering that there was no significant difference between the two years for all traits, the analyzes were performed based on the average of the two years. Data were subjected to analysis of variance (TWO-WAY ANOVA). Sources of variance were shading and no shading, potassium sulfate and sodium silicate application rates, and interactions of shading and spray treatments. Data were expressed as mean ± standard error (SE). Mean comparisons were made using LSD's test to determine if differences were significant at $p \leq 0.05$. All analyses were performed using SAS software (version 9.4). A principal component analysis (PCA) and heat map were used to correlate the data using R software (version 4.3.1).

Results and discussion

Analysis of variance

The analysis of variance revealed significant effects of shading net, foliar application, and their interactions on multiple physiological, biochemical, and fruit quality traits in pomegranate. Shading net had the strongest influence, with highly significant differences observed in photosynthesis, chlorophyll content, antioxidant enzyme activities, and sunburn incidence. In contrast, foliar treatments showed more selective impacts, while many interaction terms were not statistically significant (supplementary Tables 1, 2, and 3).

Photosynthetic parameters

Photosynthetic rate, transpiration rate, substomatal CO_2 content, leaf chamber temperature, and PAR were affected by the shading net. The highest net photosynthetic rate, transpiration, and substomatal CO_2 were observed in trees under shading net conditions (Table 1). The significant difference in photosynthetic parameters was found between the 0.15% Si and 1% K treatments, while the increase was higher for the Si application (Table 1).

Previous studies have shown that shading nets significantly affect the gas exchange status of leaves in the plants[42]. In addition, shading can lead to an increase in relative humidity, water use efficiency, vegetative growth, and leaf micromorphology[43,44]. The effects of shading nets on plant microclimate can vary according to the different shading colors and density, affecting net photosynthetic rate, transpiration rate, and water use efficiency[45]. Temperature and light intensity in the study region were significantly high in mid- summer (July and August 2020 and 2021) and at the time of the second foliar application of nutrients. In trees under open field conditions, stomata closure occurs due to high temperatures, resulting in reduced transpiration rate and CO_2 concentration below stomata, ultimately leading to reduced photosynthesis. The reduction in microclimatic temperature under shade net conditions results in reduced evapotranspiration and increased water use efficiency[45]. Pomegranate trees under full sun had higher temperatures (38 °C) than under a shade net (32 °C) (Fig. 3A) which is similar to other reports[46]. Shading nets resulted in significantly lower PAR values, 629.37 µmol m^{-2} s^{-1}, than in non-shading conditions, 1747.62 µmol m^{-2} s^{-1} (Fig. 3B). Similar results have been reported that shading nets during summer months decrease the intensity of solar radiation compared to the natural environment[47].

In general, stomatal conductance, mesophyll conductance, and water use efficiency were affected by the shade- net and mineral treatments (Table 2).

Drought stress in apple trees has been reported to reduce stomatal conductance, transpiration, substomatal CO_2 levels, and eventually photosynthesis due to stomatal closure[48]. Plants under water stress must choose between keeping stomata open for CO_2 assimilation despite water loss through transpiration or closing stomata to limit water loss and thus minimize water stress, although net CO_2 assimilation is reduced[49]. Nevertheless, Agarie et al.[50] found that Si application reduced transpiration by conserving water in leaves by closing stomata.

Levels of shading net	Level of treatments	Photosynthetic rate	Transpiration rate	Sub-stomatal CO_2
0	Control	8.61 ± 1.26d	6.05 ± 0.48ab	233.75 ± 13.42bc
0	K 1%	8.5 ± 1.31d	4.25 ± 0.35b	215 ± 7.17c
0	K 2%	9.5 ± 1.06b-d	5.66 ± 0.25ab	238 ± 17.03bc
0	Si 0.05%	9.11 ± 0.86 cd	6.05 ± 0.61ab	210.5 ± 9.95c
0	Si 0.1%	10.11 ± 0.71b-d	6.17 ± 0.66a	243.25 ± 9.56bc
0	Si 0.15%	10.43 ± 1.44a-d	6.17 ± 0.66a	253.75 ± 3.88b
50	Control	11.69 ± 1.36a-c	6.44 ± 0.82a	265.75 ± 5.96ab
50	K 1%	11.23 ± 1.77a-d	6.25 ± 0.87a	256.25 ± 3.47b
50	K 2%	12.04 ± 1.03ab	6.89 ± 0.08a	267.75 ± 0.85ab
50	Si 0.05%	11.85 ± 0.80ab	6.83 ± 1.00a	263.25 ± 6.54ab
50	Si 0.1%	12.89 ± 1.03a	7.23 ± 0.40a	267.25 ± 8.18ab
50	Si 0.15%	13.06 ± 0.96a	7.28 ± 0.48a	298.5 ± 32.23a

Table 1. Photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), Sub-stomatal CO_2 (mmol CO_2 m^{-2} s^{-1}) in control and shaded pomegranate trees. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD test ($n = 8$). Measurements were taken on 5 August at 9:00–10:00 a. m, local time.

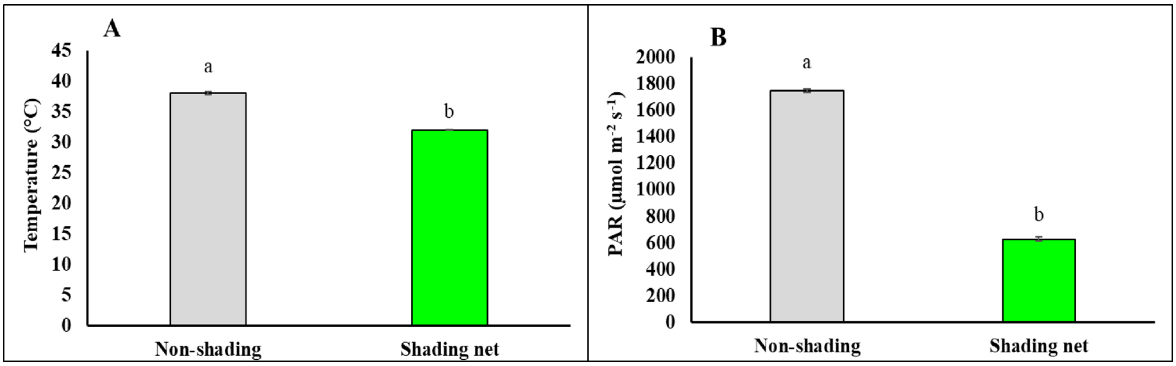

Fig. 3. Air temperature (**A**) and PAR (**B**) in control and shaded trees during 2020 and 2021 years. Means with different letters are significantly different ($P \le 0.05$) according to the LSD's test ($n = 8$). Measurements were taken on 5 August at 9:00–10:00 a.m, local time.

Treatments	Stomatal conductance	Mesophyll conductance	water use efficiency
Non-shading	0.180 ± 0.034b	0.041 ± 0.010b	1.63 ± 0.506b
Shading net	0.193 ± 0.068a	0.045 ± 0.009a	1.79 ± 0.279a
LSD	0.037	0.02	0.73
Foliar treatments			
Treatments	Stomatal conductance Mesophyll conductance Water use efficiency		
Control	0.181 ± 0.022c	0.041 ± 0.006da	1.644 ± 0.206c
K 1%	0.162 ± 0.023d	0.041 ± 0.006d	1.734 ± 0.236a
K 2%	0.175 ± 0.011c	0.043 ± 0.005c	1.723 ± 0.182ab
Si 0.05%	0.177 ± 0.037c	0.044 ± 0.004b	1.701 ± 0.247b
Si 0.1%	0.209 ± 0.024b	0.045 ± 0.004a	1.721 ± 0.124ab
Si 0.15%	0.216 ± 0.034a	0.043 ± 0.005c	1.752 ± 0.181a
LSD	0.063	0.008	0.330

Table 2. Stomatal conductance (mmol $m^{-2} \cdot s^{-1}$), mesophyll conductance (mmol m^{-2} s^{-1}), and water use efficiency (μmolco$_2$ mmol^{-1} H_2O) in pomegranate leaves under shading net and foliar spraying with K and Si. Measurements were taken on 5 August at 9:00–10:00 a. m, local time.

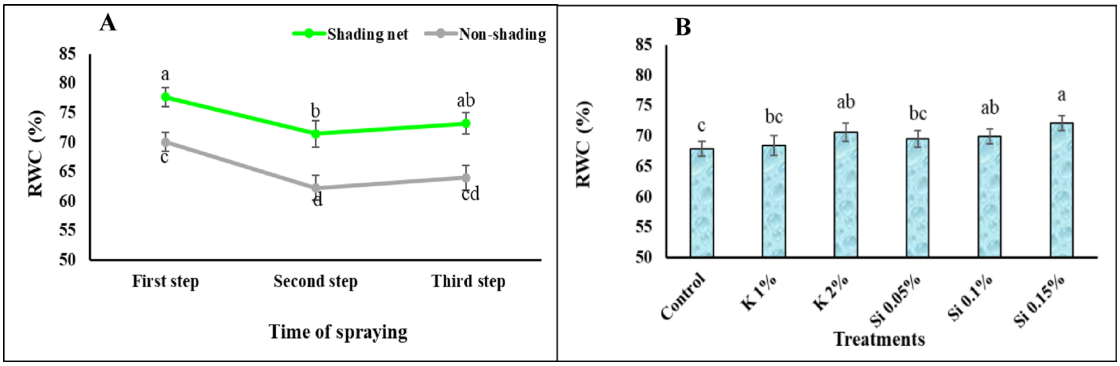

Fig. 4. RWC of pomegranate leaves under shading net (A) and foliar spraying with Si and K concentrations (B) during 2020 and 2021. Means with different letters are significantly different ($P \le 0.05$) according to the LSD's test ($n = 8$).

Leaf relative water content (RWC)

Leaves of pomegranate trees grown under 50% shaded green net had significantly higher RWC than plants grown in the open (Fig. 4A). This figure also shows that, as expected, the higher temperature and light intensity in mid-summer (second stage of nutrient application) resulted in lower leaf RWC, especially for trees grown under field conditions.

Water consumption in orchards under shade nets is significantly reduced because light intensity and temperature are reduced. According to the present results on pomegranate trees under shading nets, this may result in higher relative water content of leaves. In previous reported studies on apples, shading nets reduced potential evapotranspiration by 30%, and as less solar radiation reached the orchard canopy and soil, trees maintained higher leaf water content[44,51]. As reported by Nicolás et al.[52], due to the altered microclimate under the shading net, shaded lemon trees showed lower transpiration and higher water use efficiency.

RWC of leaves of pomegranate trees were improved by foliar application of silicon and potassium (Fig. 4B). The highest RWC was observed in the first stage, when the temperature was milder. With increasing temperature and light intensity during the season, the highest RWC was obtained at 0.15% Si (70%) and 2% K (68.69%), respectively (Fig. 4A). RWC increased with the reducing of stress conditions and after the foliar application of the third stage (Fig. 4A). In general, RWC was highest in the 0.15% Si, 2% K, and 0.1% Si treatments and lowest in the control (Fig. 4B). Consistent with the present results, Si foliar application increased RWC in cucumber leaves compared with untreated plants[53]. Under heat stress, exogenous application of Si in *Brassica napus* improved RWC as well as chlorophyll content and thus plant biomass, indicating mitigation of the negative effects of heat stress[54].

Total chlorophyll content

The highest total chlorophyll content of leaves (10 mg g^{-1} FW) was observed in the first stage and in trees grown under shade-net condition (Fig. 5A). As the season progressed to the second stage and temperature and light intensity increased, the total chlorophyll content of trees grown in the open (6 mg g^{-1} FW) decreased significantly compared to trees grown under shade net (9 mg g^{-1} FW). In the third stage of foliar application, total chlorophyll content increased compared to the second stage under shading net condition, while it decreased under field conditions (Fig. 5A).

Under the intense solar radiation, the rate of chlorophyll degradation in the fig leaf was higher than the rate of synthesis[55]. As mentioned by Taiz and Zaiger[56], leaves under shade nets have higher chlorophyll content and are generally thinner than those exposed to full sun, resulting in higher light input for photosynthesis. Consistent with the present study, an increase in total chlorophyll content of leaves was observed in citrus trees[57] and peppers[47] under colored nets. At all applied concentrations of K and Si, the total chlorophyll content of leaves increased, but the higher concentrations of K (2%) and Si (0.1% and 0.15) were significantly higher than the control treatment (Fig. 5B). Shalaby et al.[53] reported that foliar application of Si improved chlorophyll content of cucumber under combined conditions of salinity and heat stress, as is also expressed by others[58].

To protect the photosynthetic system from heat damage, energy wasting in PSII should be reduced, antioxidant enzymes should be activated to prevent the production of ROS, or ROS should be reduced to decrease its effects[59]. As reported, peroxidase and chlorophyllase activities were increased in bent grass (*Agrostis* spp.), which is a major reason for the lower chlorophyll content under heat stress[60]. Heat stress is also alleviated in plants by the increase of some metabolites or the accumulation of osmoprotectants such as soluble sugars and proline[22].

CAT, SOD and POX enzymes activity

Shading net and the supply of mineral nutrients (K and Si) had a significant effect on the activity of CAT (Fig. 6). Under the shading net conditions, the activities of CAT and SOD enzymes were significantly higher, while the activity of POX was lower in the leaves of pomegranate trees compared to the open field (Figs. 6 and 7). Compared to controls, foliar application of silicon (0.15%) significantly increased the activity of CAT under shading net (Fig. 6A). The lowest activity of CAT, POX, and SOD during the season was at the first stage of foliar spraying. The activity of these enzymes also increased significantly with increasing temperature and light intensity during the season (Figs. 6B and 7B and D).

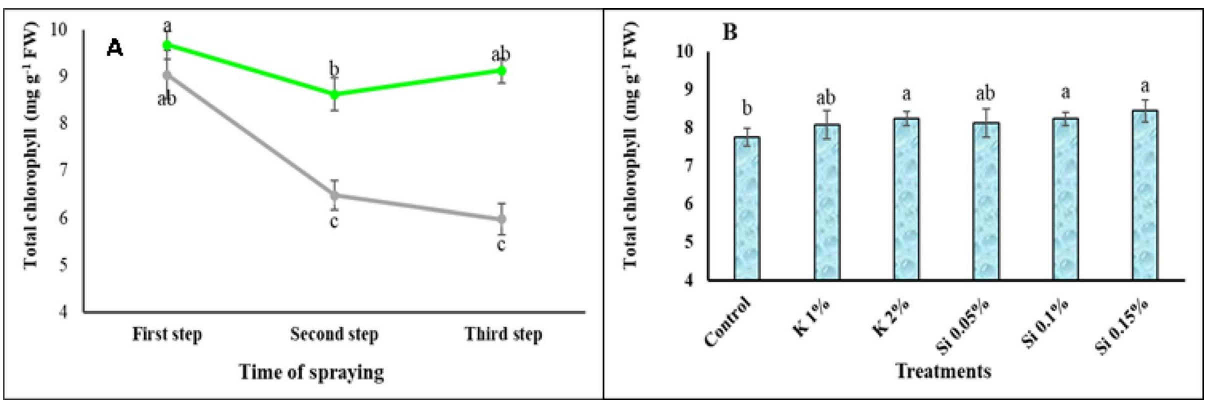

Fig. 5. Total chlorophyll content of pomegranate leaves under shading net (A) and foliar spraying with Si and K concentrations (B) during 2020 and 2021. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Samples were taken during July- September 2020 and 2021.

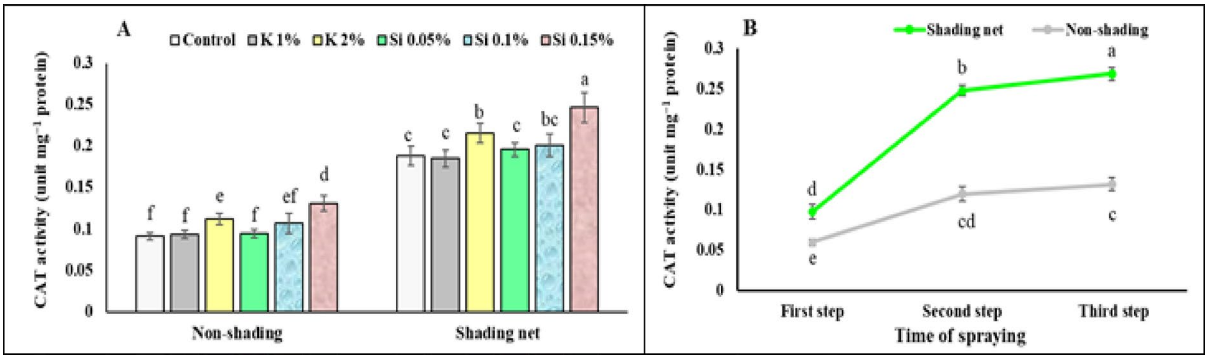

Fig. 6. CAT activity of pomegranate leaves under foliar spraying with Si and K concentrations (**A**) and shading net (**B**) during 2020 and 2021. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Samples were taken during July- September 2020 and 2021.

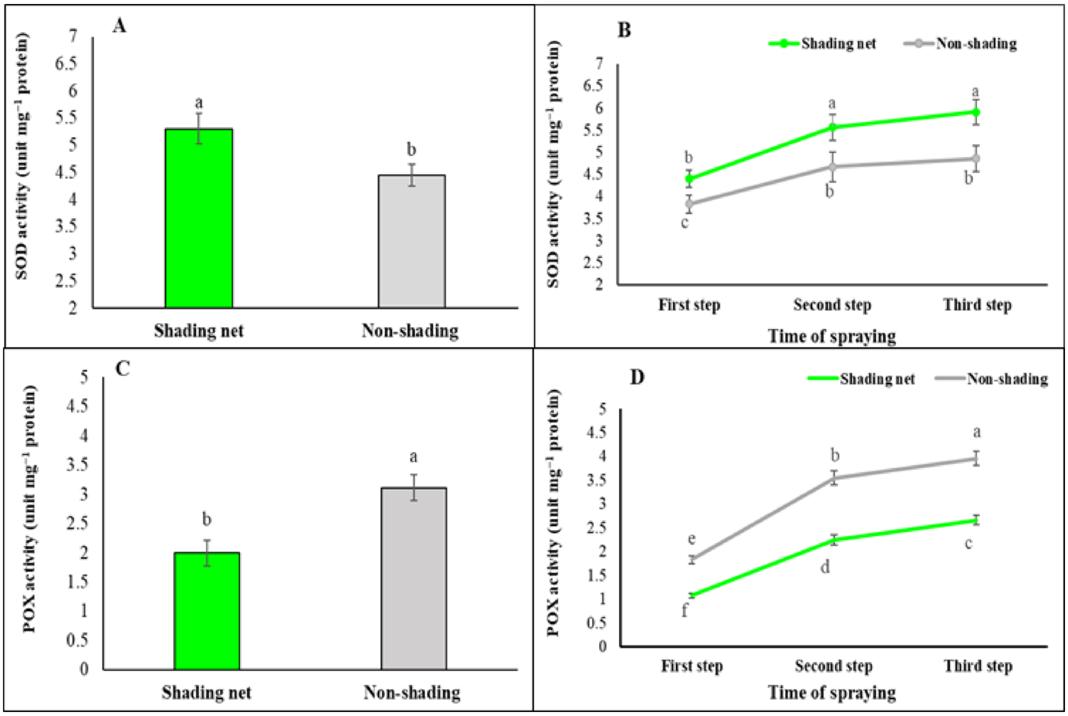

Fig. 7. SOD and POX activity of pomegranate leaves in control and shaded trees during 2020 and 2021 years. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Samples were taken during July- September 2020 and 2021.

The activity of antioxidant enzymes is considered an indicator of scavenging ROS and reducing oxidative stress[61]. The balance between ROS and antioxidant activity can be disturbed by heat stress as well as other abiotic stresses. Application of Si could enhance cell division and cell enlargement, being dependent on the physiological processes and metabolic activities such as membrane permeability, photosynthesis, transpiration, and uptake of water and nutrients[58]. Application of Si enhanced antioxidant defense systems in cucumber plants, improving their potential for tolerance under salinity and heat stress conditions[53]. Application of Si to *Salvia* under high temperature (35°C) showed that the activity of SOD increased while that of CAT decreased[26]. The role of exogenous Si applications in mitigating the adverse effects of heat stress by improving plant tolerance through increasing photosynthetic pigments, reducing oxidative stress, and lowering the level of reactive oxygen species is also documented in wheat plant[22]. Similar studies have shown that Si application enhances the ability of plants to scavenge ROS under stress conditions by regulating the activity of antioxidant enzymes[62,63].

MDA content

Shading net and foliar application of silicon decreased MDA content in pomegranate trees. Leaves of pomegranate trees under the shade net had significantly lower MDA values compared to trees in the open field (Fig. 8A). The

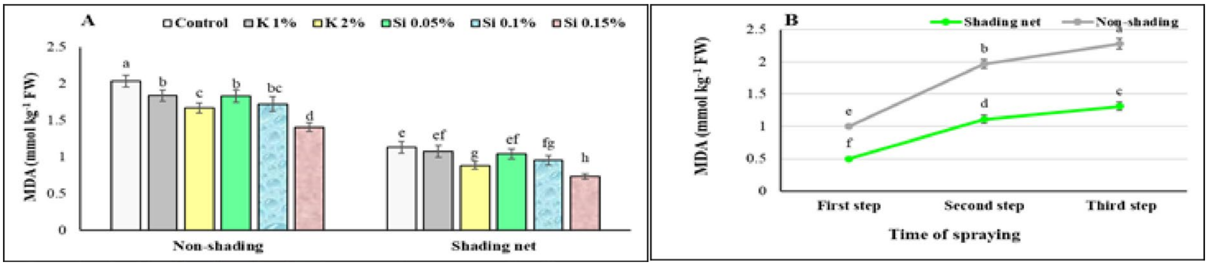

Fig. 8. MDA content of pomegranate leaves under shading net and foliar spraying with Si and K concentrations during 2020 and 2021 years. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Samples were taken during July- September 2020 and 2021.

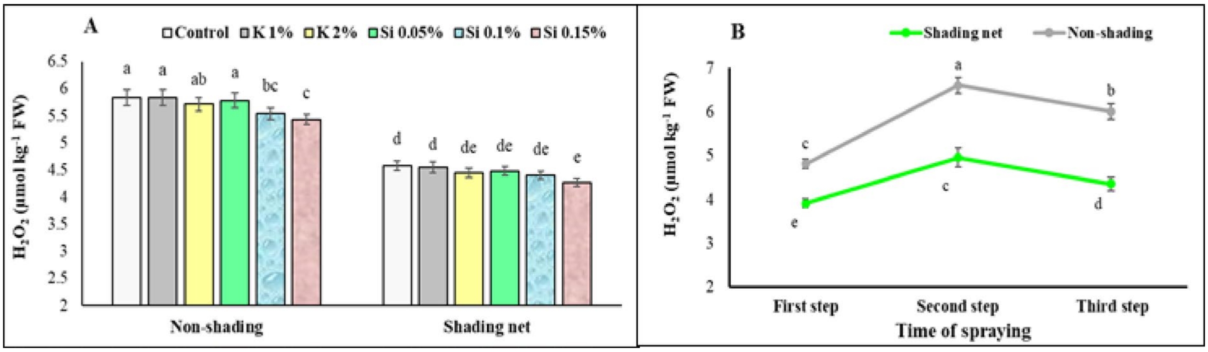

Fig. 9. H_2O_2 content of pomegranate leaves under shading net and foliar spraying with Si and K concentrations during 2020 and 2021 years. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Samples for measurements were taken during July- September 2020 and 2021.

lowest MDA content was observed in the treatments with 0.15% Si under shading net (Fig. 8A). In the second stage of leaf spraying, MDA levels increased with an increase in temperature and light intensity (Fig. 8B). Similar to the present results, application of Si was reported to significantly reduce MDA content in tomato leaves under salt stress[64]. Eraslan et al.[65] reported that application of Si reduced H_2O_2 and MDA levels in leaves of salt-stressed spinach. Similarly, foliar application of K activated the plant defense system and reduced MDA levels at moderate to high temperatures[66].

Abiotic stress, including UV-B irradiation, leads to a general disturbance of plant metabolism, causing an increase in ROS production[67]. The increased MDA content in plant tissues is due to the negative effects of ROS on peroxidation and fragmentation of unsaturated fatty acids leading to destruction of biomembrane integrity[68].

Leaf H_2O_2 content

Hyperaccumulation of reactive oxygen species (ROS) is the most important consequence of abiotic stress in plants. In unshaded pomegranate trees exposed to high temperatures and light intensity, hydrogen peroxide (H_2O_2) production was higher than in shaded trees (Fig. 9A). The effect of mineral supply itself was significant for reducing H_2O_2 accumulation. The lowest (3.80 µmol kg^{-1} FW) H_2O_2 content (5.5 µmol kg^{-1} FW) was observed in the 0.15% Si treatment (Fig. 9A). In mid-summer and with increasing temperature, H_2O_2 level in the open field and trees under the shading net increased, while it was lower in the trees under the shading net (Fig. 9B).

According to Al-aghabary et al.[64] tomato plants exposed to salt stress accumulated higher levels of H_2O_2. These results suggest that abiotic stresses, including heat stress, lead to significant metabolic changes that result to the production of ROS. The accumulation of active oxygen radicals, including H_2O_2, can result in lipid peroxidation. SOD enzyme activity is one of the major defense systems against ROS, detoxifying superoxide anion radical by converting them to H_2O_2, which is then alleviated by the activity of CAT[69]. In the present research, application of Si considerably increased the activity of SOD and CAT in leaves of heat-stressed pomegranates (Figs. 6 and 7). These results are consistent with those of Liang et al.[70] who indicated that Si application under salt stress reduced the permeability of the plasma membrane of barley leaf cells and significantly improved the ultrastructure of chloroplasts that were severely damaged by the stress. This might indicate that the damage caused by the increased production of ROS under heat stress was alleviated by the application of Si, as the activity of SOD and CAT increased and the MDA and H_2O_2 content reduced.

Fruit sunburn and cracking severity

Sunburn and cracking of pomegranate fruit, which reduce the quality of the product, are two main disorders of pomegranate fruit in the hot and dry growing regions, resulting in large losses of marketable fruit in many pomegranate producing countries. The shading net alone significantly reduced the percentage of sunburned and cracked fruit. The lowest sunburn rate (12%) and cracking rate (11%) were observed under 50% green shading net compared to the control (41% and 27% severity, respectively) (Fig. 10). Foliar application of silicon and potassium had no significant effect on fruit sunburn and cracking.

Moradi et al.[10] reported that 50% net shading resulted in significantly less sunburn and cracking on pomegranate fruit of cultivar 'Ghojagh' compared to open field. Tarabih[71] also reported that shading resulted in the lowest percentage of 'Wonderful' pomegranate fruit with sunburn (2.5%) and cracking (about 0.5%) in two consecutive experimental years compared to fruit in the open field (21% sunburn and about 9% cracking). Our results show that foliar application of potassium and silicon reduces the incidence of sunburn and fruit cracking under high temperatures. Drogoudi and Pantelidis,[72] reported that foliar spraying with calcium chloride or Si-based fertilizer containing potassium had no effect on the occurrence of physiological disorders, while Si-based fertilizer containing potassium and calcium reduced the occurrence of sunburn only in years with high damage.

The main reasons for the cracking of pomegranate fruit are variations in daily temperature and internal water content. The effect of net shading on pomegranate fruit cracking could be due to the lower heat stress on the fruit, thus reducing transpiration from the fruit surface and better retaining the water content of the fruit rind, which leads to a reduction in cracking[71]. Amarante et al.[73] reported that shading net with light reduction of 55% compared to light reduction of 15% prevents sunburn damage in apple fruit. Shading nets reduce direct sunlight on tree canopies and thus reduces sunburn[51]. According to the results of the present study and other studies, shading nets protects the fruit from intense sunlight improving external quality and reducing the incidence of sunburn and cracking in pomegranate fruit.

Leaf mineral nutrient content

The results revealed that leaf concentrations of N, P, K, Ca, Mg, and Si were increased under shading nets while Na concentrations decreased under shading (Table 3; Fig. 11), and no significant differences were found for the concentrations of Fe, Mn, Cu, and Zn (Table 3). Díaz-Pérez,[74] reported that in bell pepper leaf concentrations of N, K, Ca, Mg, S, Al, B, and Mn increased with increased shading levels for most nutrients, while concentrations of P, Cu, Fe, Mn, Na, and Zn did not show significant effect with shade level. The increased foliar nutrient concentrations observed in pomegranate in response to shading in our study are consistent with the findings on apple for N, P, and K[77]. In contrast to our results, shading treatments did not significantly affect leaf mineral contents (i.e., N, P, K, and Ca) of apple[76] and bell pepper[45].

There are few studies on leaf mineral content in fruit trees under net shading[55,75,76]. Shading net affected plant mineral concentrations, but this effect varied. The changes may be related to metabolic and structural functions[77]. Nutrients of N, Mg, Fe, and Cu are associated with various processes related to photosynthesis[75]. Nitrogen is an essential component of proteins and chlorophylls[78], Mg is the central atom of the chlorophyll molecule, and variations in its content in the chloroplast regulate the activity of important photosynthetic enzymes[79], while Fe is not only required for chlorophyll synthesis and chloroplast development but is also an important enzymatic cofactor[80]. In contrast to our results, Mészáros et al.[76] reported that net shading on apple trees did not affect Mg while limiting the uptake of N into leaves. Shading possibly ameliorated heat stress due to the changes in temperature conditions (reduction in air and root zone temperature) associated with shading, which may lead to increased mineral uptake[74]. In the present study, we did not measure the soil temperature, however canopy temperature was significantly reduced by the use of shading nets, which may be the reason for the improvement in the concentration of mineral nutrients compared to the control.

In general, foliar application of silicon increased the concentration of elements N, P, K, Ca, Mg, and Si and decreased Na, while it had no effect on Mn, Cu, Zn, and Fe (Table 3).

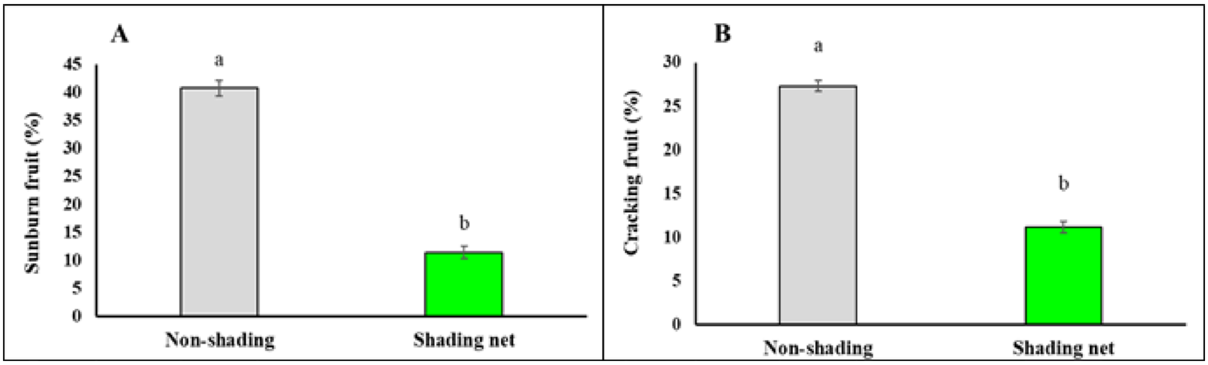

Fig. 10. Sunburn (**A**) and cracking (**B**) percentages of fruits in control and shaded trees during two years. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$). Measurements were taken in November 2020 and 2021.

Macronutrients					Micronutrients					
Treatments	**N**	**P**	**K**	**Ca**	**Mg**	**Si**	**Fe**	**Mn**	**Zn**	**Cu**	**Na**
Non-shading	17.92 ± 0.28b	14.06 ± 0.20b	6.70 ± 0.17b	14.97 ± 0.46b	3.73 ± 0.04b	0.14 ± 0.004b	72.42 ± 1.28a	41.00 ± 0.47a	11.25 ± 0.32a	6.18 ± 0.28a	1.64 ± 0.07a
Shading net	23.80 ± 0.38a	17.73 ± 0.27a	7.82 ± 0.23a	22.89 ± 0.31a	3.92 ± 0.05a	0.16 ± 0.004a	73.71 ± 1.57a	40.58 ± 0.71a	10.64 ± 0.28a	5.61 ± 0.22a	1.04 ± 0.05b
LSD	0.96	0.72	0.47	0.7	0.14	0.01	5.66	2.91	1.17	0.97	0.18
Foliar treatments											
Control	19.94 ± 0.36b	15.44 ± 0.37b	6.33 ± 0.20d	17.24 ± 0.34c	3.91 ± 0.06a	0.129 ± 0.01d	72.02 ± 4.02a	40.25 ± 1.62a	11.26 ± 0.62a	5.61 ± 0.52b	1.64 ± 0.12a
K 1%	20.02 ± 0.60b	15.44 ± 0.64b	7.40 ± 0.19bc	17.31 ± 0.38c	3.64 ± 0.07b	0.133 ± 0.01d	72.65 ± 3.48a	39.98 ± 1.10a	10.98 ± 0.83a	6.64 ± 0.50a	1.35 ± 0.09a-c
K 2%	20.49 ± 0.26b	16.08 ± 0.60b	8.22 ± 0.41a	19.41 ± 0.47b	3.83 ± 0.13ab	0.146 ± 0.01 cd	73.49 ± 2.08a	40.94 ± 1.62a	10.92 ± 0.83a	6.31 ± 0.36b	1.08 ± 0.11c
Si 0.05%	20.00 ± 0.71b	15.26 ± 0.34ab	6.44 ± 0.39d	17.73 ± 0.32c	3.78 ± 0.14ab	0.153 ± 0.01bc	72.89 ± 4.01a	40.00 ± 0.93a	10.82 ± 0.84a	6.25 ± 0.70b	1.45 ± 0.17ab
Si 0.1%	21.15 ± 0.55b	15.83 ± 0.59b	7.10 ± 0.30 cd	20.19 ± 0.40b	3.88 ± 0.06ab	0.167 ± 0.01ab	73.18 ± 3.95a	41.73 ± 1.66a	10.49 ± 0.56a	5.74 ± 0.71b	1.36 ± 0.08a-c
Si 0.15%	23.58 ± 0.51a	17.32 ± 0.42a	8.06 ± 0.28ab	21.72 ± 0.34a	3.93 ± 0.03a	0.181 ± 0.01a	74.14 ± 4.02a	41.86 ± 1.87a	11.23 ± 0.67a	4.80 ± 0.16b	1.15 ± 0.09bc
LSD	1.67	1.24	0.81	1.22	0.25	0.02	9.81	5.04	2.04	1.68	0.31

Table 3. N (mg g^{-1}), P (mg g^{-1}), K (g kg^{-1}), Ca (g kg^{-1}), Mg (g kg^{-1}), Si (g kg^{-1}), Fe (mg kg^{-1}), Mn (mg kg^{-1}), Zn (mg kg^{-1}), Cu (mg kg^{-1}), and Na (g kg^{-1}) concentrations in pomegranate under shading net and foliar spraying with Si and K concentrations during 2020 and 2021 years. Means with different letters are significantly different ($P \le 0.05$) according to the lsd's test ($n = 8$).

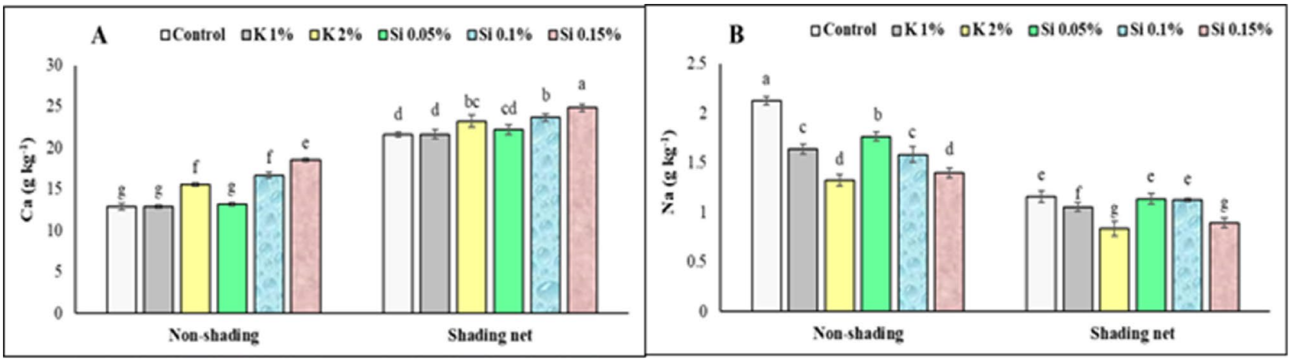

Fig. 11. Ca (g kg^{-1}) (**A**) and Na (g kg^{-1}) (**B**) concentrations in pomegranate leaves under shading net and foliar spraying with Si and K concentrations during 2020 and 2021 years. Means with different letters are significantly different ($P \leq 0.05$) according to the LSD's test ($n = 8$).

The increase in the nutrient content of the leaves may be due to the positive role of Si in improving nutrient absorption. Other researchers reported that silicon treatment increased nutrients such as Mg[83], N[84], K[85], P[86], Ca[87], Fe[88], Zn[89], Mn[90], and Cu[84] in plants under drought and salt stress.

In the present study, the highest silicon concentration in the leaf was observed for the Si 0.15 and 0.1 treatments, which were significantly higher than in the control treatment (Table 3). As reported earlier, the deposition of silica gel on leaves, stems and fruit has been associated with the beneficial effects on agricultural crops[89]. Analyses of the effects of Si supplements on macronutrient concentrations in pomegranate leaf showed differences in leaf N concentrations between the high Si concentration compared to other treatments and control (Table 3). Treatment with 0.15% Si on leaf also significantly increased P concentration compared to control and other treatments (Table 3).

El-Kareem et al.[90] reported that spraying Si improved the concentration of elements N, P, and K in date trees compared to the control. Other researchers found that Si positively affects almost all aspects of nitrogen (N) nutrition, i.e., uptake, assimilation, and remobilization[91]. In addition, Si supply can alter carbon, N and P stoichiometry in shoots[92] and increase nutrient uptake by roots[93], leading to better N utilization in plant tissues.

In addition, the Si 0.15% treatment resulted in the highest concentrations of K, Ca, and Mg and the lowest of Na (Table 3). In contrast, Abd-Alkarim et al.[94] found that foliar and soil Si treatments significantly decreased leaf K concentration compared to the untreated control in cucumber. Researchers have suggested that increased uptake of certain nutrients, such as Ca^{2+} and K^+, may be due to Si-mediated reductions in plasma membrane permeability and increased H^+-ATP activity at the plasma membrane[95]. Si could reduce abiotic stress in plants by affecting Na^+ absorption and influencing the absorption, translocation, and ultimate distribution of certain nutrient elements (via balanced nutrient uptake)[96]. Different results could be due to the involvement of Si in metabolic or physiological processes in plants, which could promote or suppress the uptake and transport of some elements depending on stress conditions[95].

Appearance characteristics of trees

Based on the results of the present study, it can be concluded that the shading net significantly reduced the harmful effects of heat stress on pomegranate trees. Figure 12 shows the appearance of the trees (leaves and fruit) under the shading net (A) and in the open field (B), coinciding with the increase in temperature and light intensity in early August. At this time, when fruit growth and development have begun, the first signs of sunburn appear on fruit grown in the open field. In August, the highest temperatures (40–45 °C) and light intensities prevail in this area. The fruit of trees grown in the open will be affected by sunburn in August, while trees under the shade net will have darker leaves and fruit with no signs of sunburn. Considering that the shading net is removed in mid-September, the pomegranate fruit still has two months to mature and ripen.

The use of shade nets in orchards has become more popular in Iran in recent years due to the very high temperatures in summer. Some nets are believed to reduce the maximum temperature during the day[11,13]. This temperature reduction benefits trees in hot climates by increasing photosynthetic rates during the hotter hours of the day and protecting fruit from sunburn[4].

Heat map clustering for the measured traits

Due to the large number of dependent and independent variables, a heat map clustering method was used to analyze and visualize the results of the present study. In the heatmap, each colored cell indicates a concentration, with colors from blue to red representing relative intensity, ranging from high to low. According to the heatmap clustering analysis, the results are highly correlated, and a clear distinction was observed in two main clusters for the treatments and measured factors. The first cluster included mainly the trees in the open field and the second cluster included the shading net. For quality parameters, POD, PAR, MDA, H_2O_2, sunburn, and cracking were assigned to the same cluster, and the levels of these parameters were higher under unshaded conditions. In contrast, activity levels of SOD and CAT, photosynthesis, chlorophyll, RWC, and macronutrients were higher in the shading net groups (Fig. 13).

Fig. 12. Pomegranate fruits and leaves under shading net (**A**) and open field (**B**) conditions. Photos taken at early August 2021.

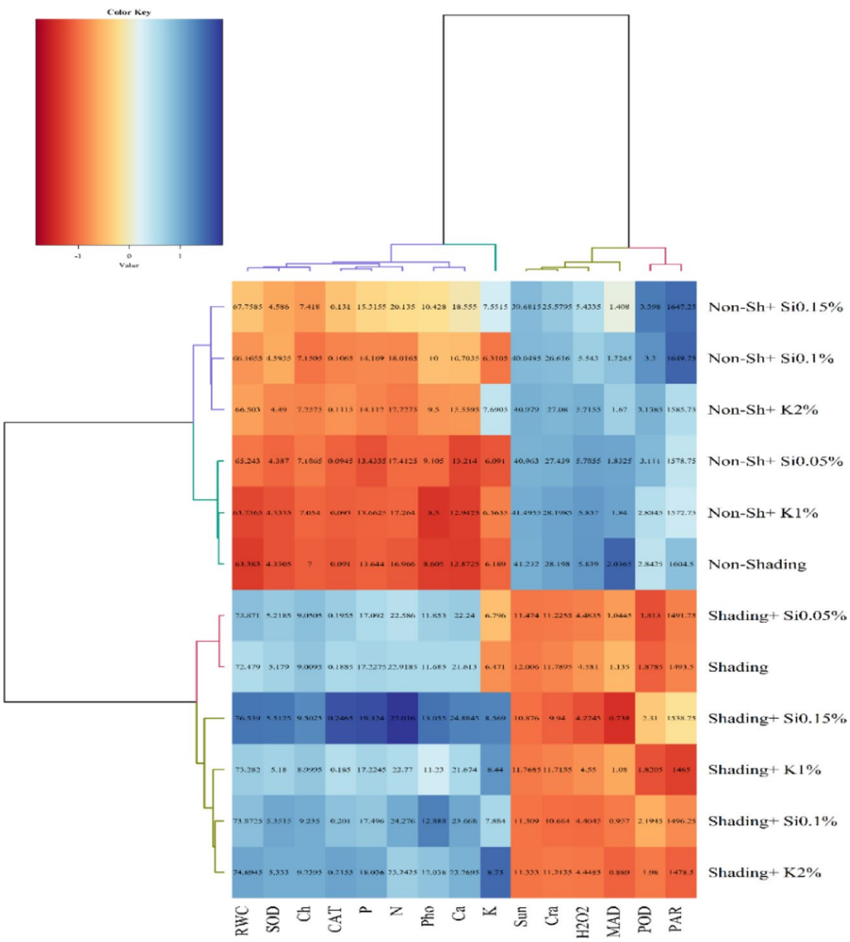

Fig. 13. Heat map clustering related to physiological and biochemical parameters (RWC, CAT, SOD, Chlorophyll, P, N, Photosynthesis, Ca, K, Sunburn, Cracking, H_2O_2, MAD, POD, and PAR) of the pomegranate tree under the application of preharvest treatments.

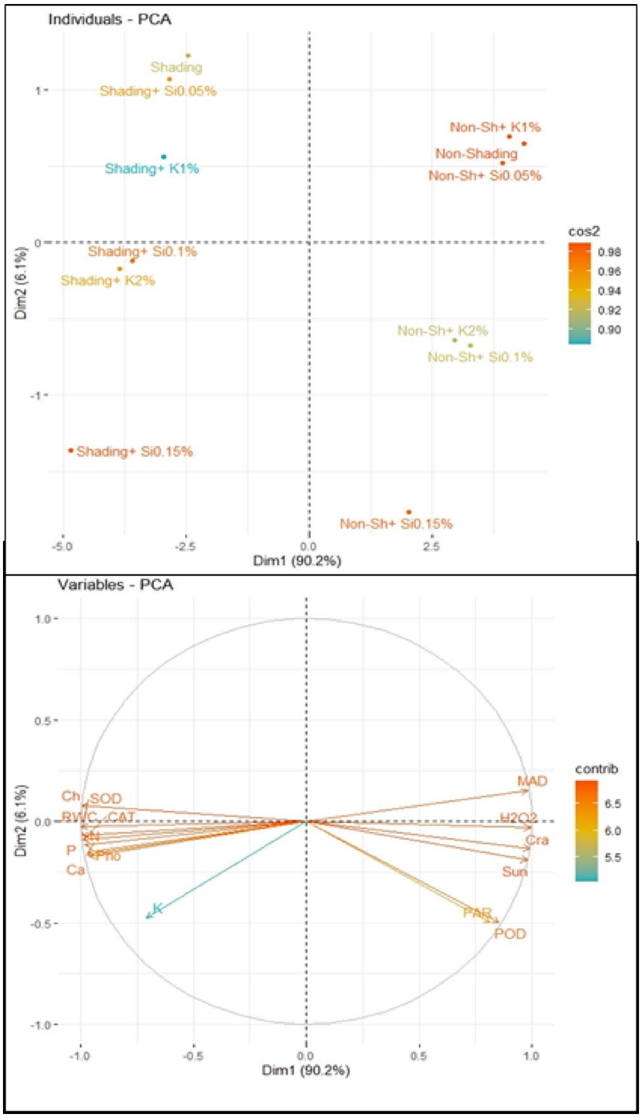

Fig. 14. PCA and Bi-Plot loading plot of traits (RWC, CAT, SOD, chlorophyll, P, N, photosynthesis, Ca, K, sunburn, cracking, H_2O_2, MAD, POD, and PAR) obtained by principal component analysis.

Bi-Plot analysis

In this study, Principal Components Analysis (PCA) was utilized to visually depict the relationship between the measured factors and the characterization of each treatment with principal components, as illustrated in Fig. 14. The extraction of principal components from eigenvalues greater than 1 yielded a variance of 96.3% within principal components for PCA dimensions 1 and 2 (PC1 (90.2%) and PC2 (6.1%)). PC1 showed a positive correlation with RWC, chlorophyll, SOD, and CAT activity, while PC2 showed a positive correlation with POD activity and PAR. The results of PCA and Bi-plot and heatmap graphs confirmed that trees grown under shading net conditions had better physiological conditions and the percentage of physiological complications was significantly reduced. Moreover, notable coefficients were observed for Chlorophyll, SOD, CAT, RWC, POD, PAR, H_2O_2, MDA, sunburn, and cracking, as depicted in Fig. 14.

Conclusion

Under conditions of high light exposure and heat stress, the use of shading nets during the growth season (from mid-June to early September) of pomegranate fruit resulted in a significant reduction in oxidative stress, as well as better fruit quality in terms of sunburn and cracking. These effects were mainly related to increased activity of antioxidant enzymes, which was accompanied by higher chlorophyll content, improved photosynthesis activity and mineral nutrients content. Exogenous supply of nutrients (Si and K) mitigated the effects of high temperatures on pomegranate trees. These nutrients, especially Si, regulated the activity of antioxidant enzymes (SOD, POX, CAT), resulting in a reduction of H_2O_2 and MDA, and improved chlorophyll content, net photosynthetic rate, and mineral nutrients. Our findings show that the effects of net shading and K and Si spraying are variable-dependent and are different among the parameters studied. According to the factor analysis, Chlorophyll, SOD, CAT, RWC, POD, H_2O_2, MDA, sunburn, and cracking showed the best response under shading net and were considered the most effective variables. The results show that the application of shading nets, especially covering the trees for a certain period from mid-June to early September, is very effective in protecting pomegranate trees from physiological disorders such as sunburn and cracking. This could be due to the effect of the nets on radiation, humidity, evapotranspiration and temperature.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions
ZZ, SM, RF and MK: Conceptualization and designing the study, methodology. ZZ: Supervision of the research, financial preparation. SM, DP: Performing the experiment, collection of data. SM and ZZ: Contribution to literature research and original draft preparation. SM, ZZ, RF, MK, VL, PI and GL: Analyzing and interpretation of data and visualization. ZZ, RF, MK, VL, PI and GL: Guiding all aspects of the research project and revising the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The authors declare that all study complies with relevant institutional, national, and international guidelines and legislation for plant ethics in the methods section. The authors declare that all that permissions or licenses were obtained to using the pomegranate trees were obtained from the University of Tehran.

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Correspondence and requests for materials should be addressed to Z.Z.