The effect of talc, kaolin, and zinc oxide on heat stress in pomegranate cv. *Malas saveh*

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Abstract

Sunburn is one of the significant problems in pomegranate growing, which reduces fruit yield and quality. This study was conducted to evaluate the impact of the application of antiperspirants on the quality and biochemical properties of pomegranate fruits. The experiment was carried out as a completely randomized design with nine treatments and three replications. Treatments included 2% talc (T) solution, zinc oxide (ZnO), kaolin (K), zinc oxide + talc (ZnT), talc + kaolin (TK), zinc oxide + kaolin (ZnT), zinc oxide + talc + kaolin (ZnTK), shading with 50% light passing, and control (sprayed with water). The highest edible fruit was measured in the ZnO treatment. The maximum and minimum thickness of fruit peel was measured in the control and shading treatments, respectively. ZnO treatment reduced the thickness of the fruit peel by 30.8% compared to the control. The lowest temperature of fruit and leaf was measured in shading and ZnT treatment, respectively. The control treatment produced the lowest Fv/Fm and leaf area (LA), and the shading treatment resulted in the highest Fv/Fm, LA, and chlorophyll. ZnO treatment increased Fv/Fm and LA by 11.95% and 15.55%, respectively, compared to the control. The highest anthocyanin and phenol of juice were recorded in shading treatment. The highest fruit peel phenol and the lowest fruit peel lipoxygenase activity were measured in the ZnTK treatment. Results indicated that the ZnO treatment is a suitable treatment due to the decrease in the percentage of fruit peel, the increase in the Fv/Fm, and the decrease in the activity of the lipoxygenase enzyme.

Keywords: abiotic stress, laccase, lipoxygenase, peroxidase, shade

Nazeri, M., S. J. Tabatabaei and N. Mollayi. 2023. 'The effect of talc, kaolin, and zinc oxide on heat stress in pomegranate cv. *Malas saveh'. Iranian Journal of Plant Physiology 14*(1), 4809-4823.

Introduction

Pomegranate (*Punica granatum*) is one of the oldest fruits grown in Iran. Edible pomegranates were first cultured in Iran during 3000 BC (Stover and Mercure, 2007). Pomegranate juice is a major source of polyphenols and has a strong antioxidant capacity (Weerakkody et al., 2010). Most of the growing area of pomegranate in Iran are located in hot and dry region where the summer sunburn is a common phenomenon. In some cases, sunburn causes the loss up to 20 to 50 percent of the total crop (Ramezani et al., 2022).

Sunburn may occur in some horticultural crops such as apples, pomegranates, pears, and grapes. Radiant sunlight, high temperatures, and water

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Received: May, 2023
Accepted: October, 2023

stress are the main factors that cause sunburn over long periods (Lotze and Hoffman, 2014). Cellular components such as proteins and nucleic acids absorb UV-B radiation, resulting in biomass reduction, impaired photosynthesis, reduced protein synthesis, and damage to DNA and other chloroplast functions. High temperatures can cause sunburn damage to the outer peel of the fruit and reduce its marketability. Under the sunburned parts of pomegranate, aril becomes dehydrated and white, which reduces the marketability of the fruit. High temperatures also make reactive oxygen species (ROS) that reduce membrane stability (Weerakkody et al., 2010). The production and elimination of ROS are related to the activity of enzymes such as peroxidase (POX).

There are several approaches to reducing sunburn disaster. The two main methods are using resistant cultivars or preventing young fruits from being exposed to sever sun light: applying proper irrigation regime and nutrition regimens that increase vegetative growth and covering the fruits with a paper bag to protect the fruit from stress condition (Kahramanoglu and Usanmaz, 2016; Moradinezhad et al., 2018). Covering the pomegranate fruits with newspaper pieces and bags in turkey was mostly not successful (Yazici and Kaynak, 2006). Another solution is to use nets, although this method is very costly (Aly et al., 2010). Another way out of this problem is reducing the temperature around the tree using sprinkler water on the tree, which is not recommended due to the scarce water sources. It is also possible to reduce sun damage using particle films, which reflect some of the light reaching the tree to reduce sunburn and improve the quantity and quality of fruit. These substances are beneficial in reducing water loss from leaves and reducing the rate of water vapor release (Rosati et al., 2006).

Kaolinite clay [$Al_2Si_2O_5(OH)_4$] based particle film was used in greenhouses and orchard systems to increase the reflection of light and reduce canopy heat, water stress, and sunburn (Shellie and King, 2013). Kaolin also reduces reactive oxygen levels and inhibits hydroxyl radicals through increasing antioxidants including phenols, flavonoids, and anthocyanin contents (Dinis et al., 2016). Zinc oxide (ZnO) is a substance used in sunscreens (Boonyanitlpony et al., 2011). large particles of ZnO create an unappealing white barrier, and it is widely used in sunscreen lotions up to 25% (Monteiro-Riviere et al., 2011).

Talc is a hydrophobic compound, hardly soluble in water. It is an indirect physical blocker of light. Talc coatings easily slide over each other, overlapping themselves and effectively increasing protective coverage on the peel (Elmarzugi et al., 2013). Foliar application of talc may protect apple fruit against pests and sunburn (Schrader et al., 2009).

This research aimed to find an effective sun light protection for pomegranate fruits using various combinations of Kaolin, ZnO, and talc, ans comparing their effects with shading method of protection.

Material and Method

This experiment was conducted in a pomegranate garden (34° 30'-N, 50° 59'-E, 925m ASL). The distance between the trees was three meters, and irrigation was carried out by flooding every 8 days. Treatments includes talc (T), zinc oxide (ZnO), kaolin (K), zinc oxide + talc (ZnT), talc + kaolin (TK), zinc oxide + kaolin (ZnT), zinc oxide + talc + kaolin (ZnTK), shading with 50% light transmission, and control (spraying with water). Foliar spraying with 2% solutions was done on two occasions: 90 days after flowering and two weeks after the first foliar application. The foliar spraying continued in the early morning until the leaves dripped. This experiment was done with nine treatments and three replications. Fruits were randomly sampled from different parts of the trees and transferred to the Faculty of Agricultural Sciences, Shahed University, Iran, in October 2017.

LA

To measure the leaf area (LA), and fresh and dry weights, 20 leaves were randomly selected from outside the crown of the tree, and their LA was measured by leaf area meter (WINAREA-UT-11).

Percentage of edible part and dry peel matter

Three fruits were selected from each tree to determine the percentage of edible and dry peel matter. Each fruit was weighed with a digital scale. Then, the edible part was separated from the peel,

and weighed with the digital scale. Percentage of the edible part was measured as follows:

$$\frac{edible\ weight}{fruit\ weight} \times 100 = Percentage\ of\ edible\ fruit$$

Then, the fruit peels were placed in an oven at 75 ℃ for 72 hours until a stable weight was reached. The dry matter percentage of the fruit peel was calculated as follows (Zamani, 1991):

$$\frac{dry\ skin\ weight}{fresh\ skin\ weight} \times 10 = Percentage\ of\ dry\ matter$$

The means of three fruits' data were calculated as the percentage of the edible part of the fruit and the percentage of the dry matter of the peel.

Peel thickness

For peel thickness, three fruits were selected, and the peel thickness was measured with a digital caliper (1114-200A China) in five parts of peels from each fruit and averaged.

Fruit and leaf temperature

A few days after the second foliar spraying, the surface temperature of 5 fruits and 10 leaves was randomly recorded by a thermometer (Testo835-T1 Taiwan) at noon to measure the temperature of the fruit and leaf.

Fv/Fm and chlorophyll index

The chlorophyll fluorescence parameter of developed leaves was recorded with a portable Chlorophyll fluorescence (Portable Fluorimeter, Hansatech, Uk Handy-Pea). After the leaf was placed in the dark with special clips for 30 minutes, Fv/Fm was measured by the device. To measure the chlorophyll index, a chlorophyll meter (Minolta SPAD-502 leaf chlorophyll, Japan) was used. The average chlorophyll of 10 leaves was calculated.

Juice extraction and fruit peel extract

Three fruits from each tree were randomly selected and fruit juice and fruit peel extract were taken by a juicer. The juice of three fruits was mixed and used to measure pH, total soluble solids (TSS), electrical conductivity (EC), total phenol, titratable acidity (TA), vitamin C, anthocyanin, and antioxidant capacity. The peel extracts of three fruits were mixed and used to determine peel pH and phenol.

TSS and EC

A digital refractometer (Hanna HI96801) was used to measure TSS. A portable EC meter (EC Tester 11) was used to measure fruit EC.

Total phenol of juice and peel

Folin Cicaltio reagent was used to measure the number of phenolic compounds in fruit peel extract (Singleton et al., 1999). Eight (8) ml of distilled water, 200 µl of sample extract, 1 ml of sodium carbonate and, 1 ml of Folin reagent solution (1:10) were transferred to the falcon. The samples remained at the laboratory temperature for 15 minutes, and the absorbance was read at 760 nm with a spectrophotometer (SHIMADZU UV-1205). The control sample without extract was obtained using gallic acid.

pH of juice and peel

To measure the pH of the fruit and peel, the juice of three fruits was taken and mixed; also, three fruit peels were extracted and mixed. Further, pH was measured with the pH meter (Clean PH500).

TA

Ten (10) cc of fruit juice was brought to a volume of 100 ml. A few drops of phenolphthalein reagent (0.5% in 80% ethanol) were added and titrated with 0.1 normal sodium hydroxide. The amount of sodium hydroxide consumed was recorded by observing the first stable pink color.

Titratable acidity was calculated using the following equation (Mazumdar and Majumder, 2003):

$$A = \frac{N \times V \times E}{M} \times 100$$

where A is the number of organic acids in fruit juice, N is the normality of sodium hydroxide consumed (0.1 normal), V is the volume of sodium hydroxide consumed, M is the amount of fruit juice (ml), and E is the equivalent weight for the

dominant acid of pomegranate (citric acid = 0.064).

Vitamin C

One ml of fruit juice was mixed with 10 ml of 5% potassium iodide. Then, three drops of 1% starch solution were added, followed by titration with 1% copper sulfate solution. The amount of ascorbic acid consumed was calculated using the following equation (Barakat et al., 1973):

Vitamin C = Volume of copper sulfate used × 0.88

Anthocyanin content

One ml of fruit extract was combined with 2 ml of 0.025 M potassium chloride buffer (pH=1). Also, another 1 ml of the extract was mixed with 2 ml of 0.4 M sodium acetate buffer (pH=5.4). The prepared solution remained at laboratory temperature for 15 minutes; then, the samples were placed in a centrifuge at 4000 rpm for 5 minutes. Finally, each prepared sample was measured separately by a spectrophotometer with 510 and 700 nm wavelengths. Total anthocyanin was calculated based on cyanidin 8-glucoside as the dominant anthocyanin of pomegranate. Ethanol was used to blank the device. The absorbance and concentration of anthocyanin were calculated as follows:

Absorbance (A) = (A 510 pH 1- A 700 pH1) - (A 510 pH 4.5- A 700 pH4.5)

Total anthocyanin (mg Kg FW^{-1}) = (A/26900) (10) (449.2) (10)

Fruit antioxidant properties assay

First, 0.008 g of DPPH was dissolved in 100 ml of ethanol. Then, 300 µl of fruit extract were mixed with 2 ml of DPPH and placed in the dark for 30 minutes. The samples were read at a wavelength of 517 nm by a spectrophotometer. Ethanol was used as a blank. Total antioxidant potential was calculated using the followinh equation (Prevc et al., 2013):

$$I = \frac{(A\ control - A\ smple)}{Acontrol}$$

Activity of lipoxygenase

Peel samples (approximately 0.1 g) were placed in a porcelain mortar and homogenized to a powder in liquid nitrogen; then, 1 ml of extraction buffer (phosphate and EDTA 0.5mM, pH=4.7) was added to it and centrifuged at 14,000 RPM and 4 ℃ for 15 min. The substrate buffer included 10 ml of linoleic acid, 4 ml of distilled water, 1 ml of sodium hydroxide (0.1N), and 5 µl Tween 80. The reaction mixture containing 180 µl phosphate buffer, 20 µl linoleic acid substrate, and 5 µl enzyme extract was transferred to a microplate. In the blank solution, phosphate buffer was used instead of enzyme extract. The reaction mixture was kept for 10 minutes in a Bain-Marie bath at 30 ℃ and finally read at 234 nm (Bonnet and Crouzet, 1977). The activity of lipoxygenase (LOX) was calculated using Beer–Lambert law using the following equation:

$$A = 25000cl.$$

Activity of peroxidase (POX)

The method of Chance and Maehly (1999) was used to measure POX enzyme activity. For this purpose, 100 mM sodium phosphate buffer (pH=7) was used to prepare enzyme extract to measure POX enzyme activity. After pulverizing 350 mg of aril tissue with liquid nitrogen and transferring it to a 2 ml tube, 1500 µl of 100 mM sodium phosphate buffer containing 2% PVPP and 1.3 mM EDTA was added to it, and then from the vortex, the samples were centrifuged at 20,000 g for 15 minutes. In order to measure the enzyme activity, 600 microliters of 100 mM sodium phosphate buffer along with 270 microliters of 2% glycol and 170 µl of 1% hydrogen peroxide were placed at 25 ℃ for 9 minutes, and then 150 µl of the enzyme extract was added to the reaction mixture. The increase in optical absorption at the wavelength of 470 nm was recorded in 3 minutes using the Elisa Reader device. The activity level of this enzyme was reported based on katal per ml of fruit extract.

Activity of laccase

First, 350 mg of aril tissue was powdered in the presence of liquid nitrogen; then, 1500 µl of 100 mM sodium phosphate buffer (pH=5) was added

Table 1
ANOVA results of the effects of various treatments of the study on physiological characteristics of pomegranate

SOV	df	Mean Square										
		percentage of edible	Percentage of peel dry matter	Peel thickness	Fruit temperature	pH of peel	Phenol of peel	LA	Fv/Fm	Leaf temperature	Chlorophy ll index	K
Treatment	8	56.43**	43.11**	3.61**	27.39**	3.49**	1.03**	120.7**	0.75**	24.83**	59.35**	44.7**
Error	18	12.79	2.62	0.12	0.47	0.001	0.00000019	20.14	0.0003	0.30	2.77	2.29
CV	-	6.33	5.62	9.84	2.51	1.08	0.45	3.71	2.34	2.23	2.8	3.38

SOV	df	Mean Square										
		pH of juice	EC	TSS	TA	Anthocyanin	Antioxidant	Phenol of juice	Vitamin C	Laccase	POX	LOX
Treatment	8	3.73**	0.77**	15.37*	0.099**	61.9**	0.64**	0.093*	1.32**	0.31**	2.99**	29133**
Error	18	0.008	0.0004	0.09	0.000008	19.54	0.0003	0.0000019	0.008	0.002	0.00007	8773293
CV	-	2.4	2.79	2.02	2.99	7.14	2.73	0.45	8.79	14.87	0.85	10.03

*** and ** indicate significance at 5% and 1%, respectively.

to it. After vortexing, the samples were centrifuged at 20,000 g for 15 minutes. Then, 600 µl sodium phosphate buffer 100 mM, 100 µl guaiacol 200 mM, and 300 µl of enzyme extract were added to the reaction mixture. The amount of guaiacol decomposition by the enzyme laccase and, as a result, the increase in light absorption at the wavelength of 465 nm was recorded for an hour by an Elisa Reader device. The activity level of this enzyme was reported based on katal per ml of fruit extract (Erkurt et al., 2007).

Potassium

Dried leaf samples were milled (Tabatabaei, 2013). To measure potassium and phosphorus, 0.5 g of powdered leaves were poured into digestion tubes. In the next step, 10 ml of nitric acid 65% was added to each tube and kept at room temperature for 12 hours. After 12 hours, the tubes containing the sample were heated to 60 ℃ for three hours and then to 110 ℃ for three hours. After cooling the tubes, 20 ml of distilled water was added to each tube and passed through filter paper to a volume of 100 ml. The amount of K in plant samples was read by a flame photometer (Prisma Tech flame photometer PTFP-5).

This experiment was conducted in nine treatments and three repetitions in the form of a completely randomized design. Statistical analysis was performed by SAS software version 9.1. Mean data were compared using Duncan's multiple range test.

Result

The analysis of the variance showed that the treatments generally had a significant effect on the measured traits at 1% and 5% probability levels (Table 1).

Fruit peel characteristics

Treatments had significant effects on the percentage of edible fruit. The highest and lowest percentage of edible fruits were measured in ZnO, and ZnK treatment (Table 2). Shading, T and ZnO treatments were similar in their effects. The maximum percentage of fruit peel dry matter was measured with 2% kaolin treatment (Table 2). The percentage of peel dry matter was also measured in shading treatment showing similar effect to the other treatments. The maximum thickness of the fruit peel, with an average of 4.77, was measured in the control. Also, the lowest peel thickness, with an average of 2.66, was measured in the shading treatment. The highest temperature of the fruit was measured with an average of 28.83 in the control treatment (Table 2). Also, the lowest temperature of the fruit surface was measured in the shading treatment with an average of 24.4. The control, ZnO, K, TK, and ZnK treatments had similar effect. The use of antiperspirants had a

Table 2
Mean comparison effect of talc, kaolin, and zinc oxide on fruit characteristics

Treatment	percentage of edible (%)	Percentage of peel dry matter (%)	Peel thickness (mm)	Fruit temperature (°C)	pH of peel	Phenol of peel (mg/l)
control	50.13±5 ab	35.42±1.11 b	4.77±0.11 a	28.83±0.6 a	3.49±0.01 b-d	0.094±0 b-d
Shade	61.56±0.82 a	23.56±0.72 c	2.66±0.33 d	24.5±0.28 c	3.43±0.02 d	0.0933±0.0003 d
T	62.8±1.66 a	26.30±0.96 bc	3.27±0.26 b-d	27.4±0.7 ab	3.44±0.02 cd	0.093±0 c
ZnO	62.83±1.63 a	26.10±1.32 bc	3.3±0.29 b-d	28±0.28 a	3.5±0.003 bc	0.093±0.0003 bc
K	50.91±0.75 bc	33.79±1.02 a	3.81±0.05 bc	28.75±0.14 a	3.5±0.01 bc	0.093±0.0003 bc
ZnT	58.67±0.64 ab	27.63±0.92 bc	2.98±0.11 cd	27.1±0.05 ab	3.47±0.01 b-d	0.094±0 b
TK	55.67±1.98 a-c	27.13±0.4 bc	3.87±0.1 bc	28±0.57 a	3.51±0.02 b	0.093±0.0003 bc
ZnK	48.65±0.26 c	29.42±0.84 b	4.09±0.18 ab	28.25±0.14 a	3.58±0.02 a	0.093±0.0003 bc
ZnTK	56.34±1.51 a-c	29.61±0.79 b	3.76±0.17 bc	25.75±0.14 bc	3.49±0.03 b-d	0.095±0 a

Treatment	LA (cm^2)	Fv/Fm	Leaf temperature (°C)	Chl. index	K (mg/g)	pH of juice	EC (ds/m)
Control	101.3±3.17 e	0.69±0.007 e	26.75±0.43 a	57.2±1.4 c	44.3±1.45 cd	3.63±0.01 bc	0.77±0.02 b
Shade	143±1.73 a	0.8±0.01 a	24.75±0.43 b	67.2±0.7 a	52.33±1.2 a	3.61±0.11 bc	0.88±0.01 a
T	148±2.3 a	0.76±0.01 a-c	26.25±0.43 a	61.5±0.5 b	40.6±0.88 de	3.74±0.05 a-c	0.76±0.01 bc
ZnO	120±3.46 bc	0.78±0.01 ab	26.65±0.2 a	53.3±1.07 d	50±0 ab	3.69±0.04 a-c	0.71±0.02 c
K	110±2.3 c-e	0.72±0.001 c-e	22.5±0.28 c	60.7±1.2 bc	41±0.57 de	3.89±0.04 a	0.78±0.005 b
ZnT	117.3±1.45 bc	0.7±0.005 de	22.25±0.14 c	63.4±0.6 ab	39.3±0.33 e	3.51±0.03 c	0.83±0.003 a
TK	126.6±1.76 b	0.72±0.003 c-e	22.8±0.11 c	61.6±1.07 b	44±1 cd	3.9±0.008 a	0.75±0.008 bc
ZnK	114.6±1.45 cd	0.75±0.01 b-d	25.75±0.43 ab	47.8±0.7 e	44±0.57 cd	3.87±0.03 a	0.74±0.005 bc
ZnTK	106±4.16 de	0.78±0.008 ab	25.8±0.11 ab	61.1±0.7 bc	47.3±0.88 bc	3.75±0.01 ab	0.75±0.003 bc

Treatment	TSS (%)	TA (%)	Vitamin C (mg/100mg)	Anthocyanin (mg/g)	Antioxidant (%)	Phenol of juice (mg/l)
control	14.96±0.03 d	0.104±0.002 c	1.29±0.058 c	70.18±4.6 b	0.65±0.013 bc	0.117±0.0005 ab
shade	16.55±0.14 ab	0.113±0.0008 b	1.69±0.04 b	88.78±0.9 a	0.53±0.004 d	0.119±0.0008 a
T	15.5±0.28 cd	0.099±0.001 cd	2.06±0.088 a	69.18±3.2 b	0.63±0.013 c	0.118±0.0006 ab
ZnO	17.13±0.08 a	0.103±0.0008 c	0.79±0.05 d	67.61±0.4 bc	0.65±0.004 bc	0.118±0.0005 ab
K	16±0.23 bc	0.094±0.002 d	1.25±0.021 c	35.75±1.1 d	0.67±0.007 bc	0.116±0.0003 b
ZnT	15.45±0.2 cd	0.136±0.001 a	0.68±0.038 d	57.58±1.9 c	0.54±0.019 d	0.117±0.0008 ab
TK	17±0.05 a	0.076±0.0008 e	1.18±0.025 c	34.53±0.9 d	0.72±0.004 a	0.116±0.0005 b
ZnK	12.74±0.19 e	0.078±0.001 e	1.62±0.076 b	63.18±1.5 bc	0.67±0.007 bc	0.117±0.0005 ab
ZnTK	13.05±0.2 e	0.092±0.002 d	1.36±0.025 c	70.3±4.1 b	0.68±0.003 b	0.116±0.0005 b

Data are mean ± standard error of three independent experiments with three replicates. T=talc, ZnO= zinc oxide, K=kaolin, ZnT= zinc oxide+ talc, TK= talc+ kaolin, ZnK= zinc oxide+ kaolin, and ZnTK= zinc oxide+ talc+ kaolin.

significant effect on the pH of pomegranate peel. The peel pH in the ZnK treatment increased by 4.18% compared to the shading treatment and 2.51% compared to the control treatment (Table 2). The highest and lowest phenol contents of fruit peel were measured in the ZnTK and T treatments, respectively. The control treatment resulted in similar effect to most other treatments (Table 2).

Leaf characteristics

The highest and lowest leaf area was recorded with an average of 148 and 101.3 in the T and control treatments, respectively (Table 2). The highest and lowest efficiency of photosynthesis was measured in shading and control treatments. Shading net increased the efficiency of photosynthesis by 13% compared to the control.

Among the foliar sprays, ZnO had the highest photosynthesis efficiency while the lowest index was measured in ZnT. The highest chlorophyll index was measured in the shading. Zinc oxide + kaolin treatment formed the lowest chlorophyll index. Different treatments had a significant effect on the amount of potassium in leaves. The highest amount of potassium was measured in the shading treatment, with an average of 52.33. Shading net treatment with ZnO resulted in similar effect. Also, the lowest leaf potassium was measured in the foliar spraying treatment with 2% T solution.

Characteristics of fruit juice

The highest fruit pH was measured in TK and ZnT treatments, respectively. Kaolin, TK, and ZnK treatments were at the same level. Shading treatment had the highest EC of the fruit. Shading increased the EC of fruit by 19% compared to zinc oxide treatment. Effects of shading treatment and ZnT were at the same level. ZnO treatment produced the highest TSS and was at the same level as TK treatment. The ZnK treatment had the lowest TSS and was at the same level as the ZnTK treatment. ZnT and TK treatments caused the highest and lowest fruit TA, respectively. TA. There was a 36% difference between the highest and lowest TA of the fruit. The foliar treatment with T and ZnT had the highest and lowest vitamin C in the fruit, respectively. Effects of K, TK, ZnTK, and control treatments were similar. The highest anthocyanins and phenols and the lowest antioxidants were measured in the shade treatment. The lowest anthocyanin and phenol and the highest antioxidant fruit were measured in the TK treatment.

Enzyme activity

The highest activity of the laccase enzyme was measured in ZnK treatment, and the lowest enzyme activity was measured jointly in the control and ZnO treatment. ZnTK, TK, and ZnT treatments were not different in their effects (Fig. I). TK and shading treatments, respectively had the highest POX activity (Fig. II). The activity of POX in TK treatment increased by 1.3% compared to the control. According to the obtained results, the lowest activity level of LOX enzyme was observed in the K treatment while the highest activity level was observed in the ZnTK treatment, so that the activity level of this enzyme decreased by 66% and 77% compared to the control and ZnTK treatments (Fig. III).

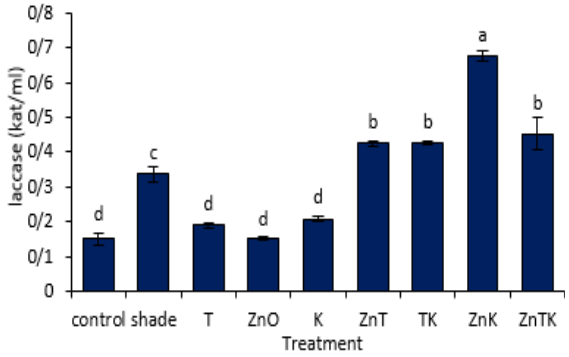

Fig. I. Comparison of mean effects of talc, kaolin, and zinc oxide on laccase activity; T=talc, ZnO= zinc oxide, K=kaolin, ZnT= zinc oxide+ talc, TK= talc+ kaolin, ZnK= zinc oxide+ kaolin, and ZnTK= zinc oxide+ talc+ kaolin.

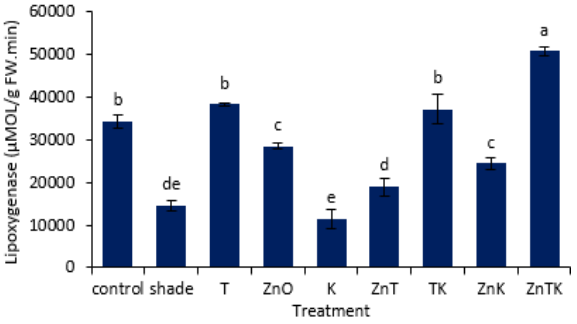

Fig. II. Comparison of mean effects of talc, kaolin and zinc oxide on POX activity; T=talc, ZnO= zinc oxide, K=kaolin, ZnT= zinc oxide+ talc, TK= talc+ kaolin, ZnK= zinc oxide+ kaolin, and ZnTK= zinc oxide+ talc+ kaolin.

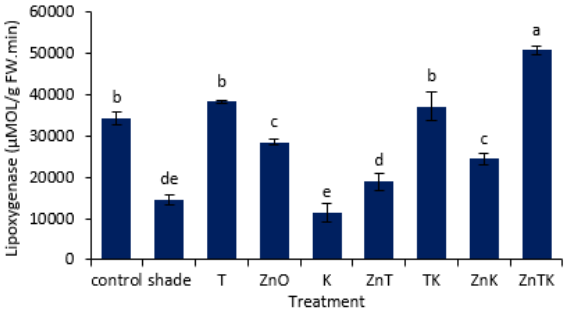

Fig. III. Comparison of mean effects of talc, kaolin and zinc oxide on LOX activity; T=talc, ZnO= zinc oxide, K=kaolin, ZnT= zinc oxide+ talc, TK= talc+ kaolin, ZnK= zinc oxide+ kaolin, and ZnTK= zinc oxide+ talc+ kaolin.

Table 3
Correlation coefficient between the traits under study

	TSS	pH	Ec	Vitamin C	Antioxidant	TA	Anthocyanin	Phenol of juice	pH peel	Phenol of peel	percentage of edible
pH	-0.458*	1									
Ec	1	-0.458*	1								
Vitamin C	0.055	0.267	0.055	1							
Antioxidant	-0.774**	0.743**	-0.774**	0.065	1						
TA	0.601**	-0.808**	0.601**	-0.374*	-0.855**	1					
Anthocyanin	0.306	-0.53**	0.306	0.342*	-0.525**	0.357*	1				
Phenol of juice	0.464*	-0.524**	0.464*	0.351*	-0.558**	0.397*	0.45*	1			
pH peel	-0.503**	0.457*	-0.503**	-0.120	0.517**	-0.515**	-0.362*	-0.248	1		
Phenol of peel	0.058	-0.105	0.058	-0.333*	0.121	0.028	-0.047	-0.394*	0.002	1	
Percentage of edible	0.069	-0.203	0.069	0.112	-0.238	0.277	0.164	-0.314	-0.436*	-0.321	1
Peel thickness	-0.493**	0.427*	-0.493**	0.011	0.612**	-0.501**	-0.301	-0.457*	0.425*	0.179	-0.617**
Percentage of peel dry matter	-0.189	0.201	-0.189	-0.141	0.367*	-0.183	0.091	-0.314	0.304	0.403*	-0.623**
Chlorophyll	0.706**	-0.314	0.706**	0.057	-0.478*	0.442*	-0.359	0.215	-0.666**	0.212	0.389*
Fv/Fm	0.096	0.029	0.096	0.198	-0.094	-0.074	0.523**	0.075	-0.103	0.162	0.265
LA	0.299	-0.101	0.299	0.496**	-0.367	0.102	0.286	0.541**	-0.469*	-0.568**	0.637**
Leaf temperature	-0.394*	-0.110	-0.394*	0.351*	0.147	-0.169	0.655**	0.108	0.059	-0.141	0.035
Fruit temperature	-0.587*	0.389*	-0.587**	-0.234	0.535**	-0.283	-0.569**	-0.390	0.449*	-0.202	-0.252
LOX	-0.429*	0.001	-0.429*	0.164	0.399	-0.235	0.192	-0.294	-0.015	0.447*	0.046
Laccase	0.172	0.061	0.172	0.069	-0.107	-0.091	0.075	-0.063	0.301	0.251	0.637**
POX	-0.103	-0.027	-0.103	-0.016	0.104	-0.077	0.075	-0.312	0.145	0.5**	-0.091
K	0.103	-0.084	0.103	0.04	-0.105	-0.051	0.536**	0.152	-0.064	-0.036	0.266

	Peel thickness	Percentage of peel dry matter	Chl.	Fv/Fm	LA	Leaf temperature	Fruit temperature	LOX	Laccase	POX
Percentage of peel dry matter	0.722**	1								
Chlorophyll	-0.5**	-0.241	1							
Fv/Fm	-0.381*	-0.449*	0.081	1						
LA	-0.557**	-0.729**	0.393*	0.176	1					
Leaf temperature	0.237	-0.017	-0.443*	0.314	0.026	1				
Fruit temperature	0.237	0.549**	-0.551*	-0.65**	-0.424*	0.017	1			
LOX	0.528**	0.073	-0.047	0.202	-0.147	0.502**	-0.078	1		
Laccase	0.267	-0.141	-0.167	0.223	-0.099	-0.19	-0.424*	0.017	1	
POX	0.167	0.19	0.058	0.316	-0.396*	0.174	-0.198	0.467*	0.059	1
K	-0.186	-0.361*	0.009	0.739**	0.117	0.424*	-0.445*	-0.019	-0.085	0.152

* and **: significant at 5% and 1% probability levels, respectively

Correlations

Finally, correlation results (Table 3) showed a negative relationship between edible fruit with peel thickness and peel dry matter percentage and a positive relationship with chlorophyll index and LA. Also, TSS and EC negatively correlated with leaf and fruit temperature.

Discussion

The percentage of the edible part and the peel thickness of the pomegranate are the most important characteristics of a pomegranate. The structural features of the peel, such as thickness and cell wall structure, are cultivar-specific and genetically influenced (Rolle et al., 2015).

Auxin is formed from the amino acid tryptophan, which is synthesized in the presence of zinc. Research has shown that auxin causes hydrogen ions to enter the cell wall and lowers the pH, destroying the cell wall xyloglucan and cellulose bonds. The reduction in the thickness of fruit peel and the percentage of peel can also be the reason. Research has shown that using 25, 50, and 75 g/l kaolin decreased the fruit thickness in mango (Baiea et al., 2018). However, with increasing concentration and frequency of foliar kaolin, the peel thickness in Balady mandarin increased (Ennab et al., 2017). Exogenous application of 5%

(w/v) kaolin on grapes increased the percentage of peel compared to seed (Luzio et al., 2021). Using kaolin particle film reduced the leaf thickness in gooseberry under full irrigation and drought stress (Segura-Monroy et al., 2015). As a result of increasing the fruit's temperature, the evaporation of water from the surface of the fruit increases. Consequently, soluble nutrients in water reach the fruit in a greater amount and increase the thickness of the fruit peel. A correlation of 0.528 was observed between fruit temperature and fruit peel thickness.

The most important way to reduce plant temperature is water evaporation. Unlike leaves, many types of fruits have minimal cooling capacity via transpiration from the fruit's peel (Schrader et al., 2003). Nevertheless, this method is the most crucial way to reduce the temperature of the fruit. The transport of nutrients in plants is through the xylem and phloem and in the form of water solution. Any part of the plant has a higher temperature, the evaporation and transpiration from that area are more. Also, an increase in fresh and dry peel weight in the control treatment can be due to the increased evaporation from the surface of the fruit and the accumulation of substances in the fruit's peel. Research has shown that kaolin increased the dry matter of the physalis (Segura-Monroy et al., 2015), and in this experiment, the dry weight of the leaves increased in the kaolin treatment compared to the control.

When particle films are applied to the fruit, there is sufficient residue on the surface of the fruit. This residue lowers the temperature of the fruit and, consequently, the solar lesion. Zinc oxide has photocatalytic properties (Li et al., 2011). In addition to UV light, high temperature is also one of the sources of sunburn (Glenn et al., 2002). The coating of kaolin particles reduces the surface temperature of the fruit through the reflection of visible light, infrared rays, and ultraviolet effect (Glenn et al., 2001). The decrease in temperature depends on the concentration and times of sprayed kaolin. By increasing the number of times of foliar spraying through Surround® WP (a commercial light reflective material manufactured based on kaolin) the surface temperature of apple fruits decreased on August 7, 8, and 9 (Glenn et al., 2002). As a result of the kaolin foliar application, the fruit temperature decreased slightly. This decrease can be due to low concentration or low frequency of foliar spraying. Foliar spraying of physalis with 5% kaolin (w/v) decreased the leaf temperature by 1.4 ℃ (Segura-Monroy et al., 2015). The process of reducing the temperature of the leaves generally involves an increase in the reflectance of the light of the treated leaves, which reduces the amount of light they absorb.

PSII can be used as an essential indicator to determine the amount of stress in a plant. The Fv/Fm ratio shows the maximum quantum efficiency of the PSII and is an important parameter to determine the state of the photosynthesis system (Krause and Weis, 1991). The reduction of PSII quantum efficiency is due to damage to PSII reaction centers. Similar research on grapevine showed that foliar spraying with kaolin 5% (w/v) increased Fv/Fm (Dinis et al., 2018). UV-B radiation and high temperature damage chloroplasts, particularly PSII and indirectly generate ROS that can further damage the photosynthetic apparatus (Mittler, 2002; Takahashi and Badger, 2011).

A correlation of -0.514 was found between leaf temperature and photosynthesis efficiency (Table 2). Research has shown that 2% kaolin spray on cellophane coating reduces UV light by 40.5% (Emarlo, 2018). The effects of adding nano zinc oxide in talc powder have been investigated. UV light transmission decreased with the addition of nano zinc oxide in talc powder. Also, research has shown that zinc oxide nanoparticles 5% absorb 91% of UV-A and 100% of UV-B, and their UV transmission rate is 0.09% (Abdullah et al., 2011). This might explain why chlorophyll index was not reduced in ZnT. Spraying *Physalis peruviana L.* with kaolin was shown to increase the efficiency of photosynthesis and chlorophyll index (Segura-Monroy et al., 2015), which is the similar to the results of this study.

With the increase in light intensity, the temperature of the leaf also increases, and as a result, the evaporation of water from the leaf surface increases. Reduction of plant growth is the first reaction of the plant to drought stress. Cellophane coated with 2% talc solution reduced

the passing PAR by 2% (Emarlo, 2018). A correlation of -0.526 was obtained between LA and leaf temperature (Table 2), which indicates a decrease in LA due to an increase in leaf temperature. During fruit growth, there is a competition between fruit and leaf to absorb water and nutrients. Moreover, as the fruit's temperature increases, the competition for water absorption increases. A correlation of -0.424 between fruit temperature and LA shows that LA decreases with increasing fruit temperature.

Potassium absorption takes place by mass flow (Marschner, 2012). In plant leaves, potassium absorption is stimulated by light (Zörb et al., 2014). The opening and conduction of stomata are influenced by blue light, UV-A, and UV-B (Eisinger et al., 2003). Research has shown that foliar spraying with kaolin reduces UV-A and UV-B (Glenn and Yuri, 2013). Research has shown that commercial samples of talc absorbed 62% of UV-A and 93% of UV-B (Abdullah et al., 2011). As a result of reducing stomatal conductivity by kaolin, transpiration is reduced, and the absorption of water and elements by mass flow is reduced. These materials prevent the loss of water from leaves by creating a layer on the leaf, reducing the leaf temperature, and preserving the plant's water potential. Also, using kaolin in coffee trees decreased the amount of potassium (Steiman et al., 2011).

Phenols are widely present in plants and are the most abundant secondary metabolites in them. The production and stability of phenolic compounds depend on the degree of fruit ripening and temperature, light, and enzyme activity. Plants have developed a variety of mechanisms for avoiding the damaging effects of UV rays. One of them is quenching ROS with flavonoids and other phenolic compounds (Agati et al., 2012). The reduction of total phenols in T treatment can be due to the absorption of UV light by talc (Abdullah et al., 2011). As the fruit ripens, phenolic compounds increase (Weerakkody et al., 2009). The number of phenolic compounds in the peel of Golden Delicious apples outside the tree crown and sunburned compared to apples inside the tree crown increased by seven percent (Zupan et al., 2014). The reduction of phenol in the shade net treatment can be due to the lack of ripening of the fruit or the reduction of photosynthetically active radiation, which results in the reduction of PAL enzyme activity and phenol production. Using commercial compounds based on kaolin also increased the number of phenolic compounds in the pomegranate variety Kandahari by 29% compared to the treatment without foliar spraying (Sharma et al., 2018).

pH has a direct relationship with the amount of sugar and indirectly correlates with organic acids; with increasing respiration, acidic compounds are converted into simple sugars. Zinc oxide, with its photocatalytic properties, breaks down organic acids (Li et al., 2011). The use of zinc oxide nanoparticles at a concentration of 1.25 grams per liter increased the pH in grapes and strawberries by 3% (Emamifar and Mohammadizadeh, 2015; Emamifar, 2018). In other studies, there was no significant difference in the pH of pomegranate juice sprayed with 2.5% kaolin compared to the control (Ehteshami et al., 2012).

Kaolin foliar spray decreased leaf temperature and increased leaf water potential. Increased photosynthesis has led to an increase in soluble proteins, soluble sugars, and starch. Similar research on grapevine showed that foliar spraying with kaolin 5% (w/v) increased soluble sugars (Dinis et al., 2018). Sharma et al. (2018) reported development in TSS and juice betterment of 'Kandhari' pomegranates after Surround® WP sprays. Also, research indicated that spraying mango with kaolin at concentrations of 25, 50, and 75 g/l increased TSS and TA (Baiea et al., 2018). Kaolin foliar application of 25 g/l on Keitt mango showed that the amount of vitamin C was not significantly different compared to the control (Baiea et al., 2018).

As previously mentioned, phenylalanine ammonia-lyase (PAL) is a key enzyme at the beginning of the phenylpropanoid pathway, running the substrate for the activity of chalcone synthase, which marks the beginning of the flavonoid biosynthesis pathway, in which, among other compounds, anthocyanins are synthesized (Dinis et al., 2016). Research has shown that light stimulates the PAL enzyme. On the other hand, high light intensity destroys anthocyanin (Bamneshin et al., 2022; Zhang et al., 2012). The

decrease in the amount of anthocyanin in the control compared to the shading treatment can be attributed to the destruction of anthocyanin due to high light intensity.

Laccase and POX are two important enzymes in lignin formation, and the activity of these two enzymes was shown to decrease in cells where lignin formation occurred (Koutaniemi et al., 2007). Peroxidases are among the antioxidant enzymes that play a significant role in suberization, lignification, response to environmental stresses, and cross-linking of the cell wall. Peroxidase provides the necessary substrate for lignin by taking electrons from phenol to reduce hydrogen peroxide (Gaspar et al., 1985).

The most important mechanism of plants to deal with high temperature and light stress is the production of antioxidant substances, and plants exposed to stress regulate POX activity. The activity of antioxidant enzymes increases with increasing temperature. As the amount of these substances decreases, the damage resulting from the production of active oxygen increases (Aly et al., 2010). Regulation of antioxidant cycles such as POX has been confirmed in response to stresses. In addition, POX is also involved in repairing damaged tissues, cross-linking mechanisms, and cell wall attachment, which contributes to the plant defense system (Passardi et al., 2005). Peroxidase enzyme activity in the peel of sunburned apples increased by 60% compared to those in the shade (Zupan et al., 2014). Increased POX activity in TK, ZnK, and ZnTK treatments can indicate the increase in POX production in these treatments. In other studies, it has been shown that the activity of anti-oxidation substances is increased in pomegranate aril and peel sprayed with 2% and 4% kaolin (Falahi, 2013). Laccase increases lignin formation in plants (Pourcel et al., 2005). So far, there has been no research on the effect of kaolin, zinc oxide, and talc on the laccase enzyme in trees and fruits. Laccase has been used to remove colored compounds (phenolic and non-phenolic) from underground water, and mineral oxides such as zinc oxide cause immobility of laccase and color decomposition (Rani et al., 2017). In this experiment, ZnO treatment had one of the lowest levels of laccase enzyme. Laccase is part of the lignin synthesizing system in wood tissues and, together with POX, causes lignification. Laccase is one of the first enzymes capable of polymerizing lignin monomers. Hydrogen peroxide needed by laccase for this process is provided by the peroxidase enzyme (Gaspar et al., 1985).

Lipoxygenase catalyzes the peroxidation of unsaturated fatty acids from the cell wall. The release of fatty acids provides the necessary substrate for the enzyme LOX. Peroxidation of membrane fats causes ACC to become available to ACC-oxidase, thereby increasing ethylene production and intensifying plant cell aging (Bhattacharjee, 2005). Lipoxygenase attacks the cell membrane and initiates membrane lipid peroxidation. In an experiment on the effect of Surround® WP on pomegranate var Kandahari and apple var Delicious, the LOX enzyme activity decreased by 35% and 26% compared to the treatment without foliar spraying (Sharma et al., 2018; Sharma et al., 2020).

Conclusion

According to the obtained results, shading is more effective than other treatments to mitigate sunburn in pomegranate fruits, but due to the high cost of installing netting, using evaporation and transpiration-reducing materials is recommended for this purpose. Accordingly, foliar spraying of trees treated with ZnO decreased peel thickness and increased the percentage of edible part of the fruit, Fv/Fm, LA and TSS. This treatment reduced the activity of LOX enzyme and increased leaf potassium contents.

Acknowledgements

This work was supported by the Planning and Budget Organization of Islamic republic of Iran. We would like to express special thanks to Dr. Babalar and Fatahi for guidance in writing the article.

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