ACTA SCIENTIARUM POLONORUM Acta Sci. Pol. Hortorum Cultus, 21(3) 2022, 131–140

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.3.12

ORIGINAL PAPER

Accepted: 28.10.2021

EFFECT OF SALICYLIC ACID AND KAOLIN ON YIELD, PHYSIOLOGICAL TRAITS, AND FATTY ACID COMPOSITION IN OLIVE CULTIVARS UNDER REGULATED DEFICIT IRRIGATION

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ABSTRACT

In this study, effect of salicylic acid (SA) and kaolin (KL) separately and simultaneously on yield, physiological traits and fatty acids composition of two olive cultivars (i.e. Zard and Roghani) was investigated under three irrigation regimes. Results showed that deficit irrigation, especially 50% crop evapotranspiration (ETc), increased accumulation of MDA, proline and activity of antioxidant enzymes, but decreased chlorophyll content and yield in both the olive cultivars, as compared to full irrigation (100% ETc). Applying SA and KL alleviated the harmful effect of water deficit on plants through enhancing their chlorophyll content and antioxidant activity, and accordingly improved their yield, as compared to the controls. On the contrary, low irrigation mounted some fatty acids such as palmitoleic acid, stearic acid, arachidic acid, while reduced unsaturated/saturated fatty acids. Moreover, applying SA and KL simultaneously, as compared to the controls, increased unsaturated/saturated fatty acids and the quality of extracted olive oil. In general, the results showed that a simultaneous effect of SA and KL, in comparison to their separate application, had a better effect on yield and quality of olive under water deficit.

Key words: catalase, evapotranspiration, malondialdehyde, oil quality, proline

INTRODUCTION

Olive (*Olea europaea* L.) is an evergreen and drought-tolerant plant and one of the most important plants native to Mediterranean regions; its fruit is widely used in processing and obtaining oil [Brito et al. 2018a]. The quality and composition of olive oil are affected by some environmental factors such as temperature, light intensity, wind, geographical altitude, water availability, genetic factors, and stage of fruit ripening [Khaleghi et al. 2015, Brito et al. 2019b]. As cited above, olive usually is native to dry and semi-dry regions with Mediterranean climate. In these regions, water deficit and high temperature are prevalent, and they negatively influence olive's growth and productivity [Doupis et al. 2013, Gholami and Zahedi 2019b]. Although olive naturally tolerates drought condition [Petridis et al. 2012], studies showed that

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drought had a negative effect on its growth, yield, and photosynthesis therefore, performing an irrigation improves its yield under this condition [Petridis et al. 2012, Brito et al. 2018a].

Using some methods efficient in reduction of plants' transpiration could culminate in attaining a better olive tolerance to water deficit. In this regard, KL- $Al_2Si_2O_5(OH)_4$- is characterized by its anti-transpiration property [El-Deen et al. 2015]. It is a white and chemically neutral mineral with a high light-reflecting property, called particle film technology [Cantore et al. 2009]. Spraying plants' surface with a KL's liquid solution can induce their tolerance to drought [Glenn et al. 2005]. The accumulation of KL particles on the surface of leaves improves some issues such as reflecting sunlight, implanting a change in angle of irradiance, making a balance in plants' temperature, and reducing the damage exerted to leaf and fruit under higher temperatures [Glenn 2012, Khaleghi et al. 2015].

As an endogenous growth regulator derived from natural phenol compounds, salicylic acid (SA) – orthohydroxy benzoic acid – regulates some physiological processes such as flowering induction, plants' growth and development, ethylene biosynthesis, adjusting stomatal conductance and respiration [Raskin 1992]. By changing the activity of antioxidants enzymes, SA negates harmful effects of oxidative reactions and induces plants tolerance towards environmental stresses [Hernández et al. 2017]. A great deal of information is available regarding separate effect of KL and SA on mitigating the deleterious effect of environmental stresses on the plant [Noreen et al. 2010, Khaleghi et al. 2015, Brito et al, 2018a, 2019a], but no information is found on their simultaneous effect on water deficit, and this prompted us to investigate the separate and simultaneous effects of SA and KL on tolerance of Zard and Roghani olive cultivars towards water deficit under field condition.

MATERIALS AND METHODS

Plant material and treatments

This experiment was performed in a research station of Dalaho olive located in Sarpol-e-Zehab Kermanshah province, Iran. The plant material of this research included 16-year-old trees of the two olive cultivars (i.e. Zard and Roghani). Soil physical and chemical characteristics are shown in Table 1. This experiment was laid out as a factorial arranged in a randomized complete block design with three replicates. In this research, the experimental factors included foliar spraying (distilled water – control; 1 mM SA, 2.5% KL – WP, Sepidan, Iran or combination of SA and KL in mentioned concentrations), irrigation regimes (100%, 75% and 50% ETc), and olive cultivars (i.e. Zard and Roghani). SA and KL was applied in form of foliar spray with a mean volume of 5 l for each tree. The trees planted in 6 × 6 m distance and each unit of trail included 2 trees. The rate of potential transpiration and trees' water requirement (from early of May till early November – onset of rainfall) was calculated using daily data of metrology provided by the synoptic station of Sarpol-e Zahab and using Penman Monteith equation (ETo Calculator Software). Based on the rate of transpiration and water requirement of the olive cultivars as well as regarding olive's crop coefficient estimated previously, the irrigation was scheduled and irrigation applied by drip system at a three-day interval. The olive trees were fertigated every year by a nutrient compound containing 100 kg ha^{-1} N, 120 kg ha^{-1} K_2O and 60 kg ha^{-1} P_2O_5. Also, pest and weed control, were performed according to the local farming practices.

Table 1. Physical and chemical characteristics of the soil in Sarpol-e Zahab Olive Station

Soil depth (cm)	Silt (%)	Sand (%)	Absorbable potassium (ppm)	Absorbable phosphorus (ppm)	Total nitrogen (%)	Organic carbon (%)	$CaCO_3$ (%)	pH
0–30	44	24	348	12.67	0.24	1.93	34	7.35
30–60	37	27	112	7.1	0.05	1.19	37	7.55

Measuring the traits

Proline. Proline content was measured based on the method introduced by Bates et al. [1973]. To do so, 0.3 g of fresh leaf was homogenized with 10 ml of 3% sulfosalicylic acid (W/V) using mortar and pestle. Then, the homogenate was centrifuged at 10 000 rpm for 15 min. 2 mL of the extract was added to 2 mL of ninhydrin regnant and 2 mL of acetic acid. Then, the resultant was placed in a bath water at 100°C for 1 h. After cooling the mixture by placing it in ice water to stop reaction, 4 ml of toluene was added and well shaken to push proline going into toluene phase. Finally, the extracts left for 30 min, then the absorbance of supernatant was read at 515 nm using a spectrophotometer and toluene was used as control (blank). The rate of proline in leaves of the olive was expressed as micromole per fresh weight of leaf.

Chlorophyll content. For measuring chlorophyll, 0.1 g fresh leaf was ground with 5 ml of aqueous 80% acetone. Then, the extract was centrifuged at 3000 rpm for 10 min. Then, 1 ml of supernatant was added to 4 ml of 80% acetone. The absorbance of samples was read at 663 and 645 nm using a spectrophotometer [Strain and Svec 1996]:

Malondialdehyde. In order to measure MDA content, 0.2 g fresh leaf was homogenized in 5 mL 1% trichloroacetic acid. The homogenate was centrifuged at 5000 rpm for 5 min. Then, 5 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid (TBA) was added to 1 mL of aliquot of the supernatant, and kept in a water bath at 100°C for 30 min. Then, it was quickly cooled in an ice bath. The absorbance of the supernatant was read at 532 nm. The value for the nonspecific absorption at 600 nm was subtracted from the 532 nm reading. The rate of MDA was calculated based on Nano mole per fresh weight of leaf [Stewart and Bewley 1980].

Antioxidant enzyme activity. The fresh leaf was homogenized with 2ml of 50mM phosphate buffer (pH 7.0) containing 1mM EDTA and 2% PVP (w/v). The homogenate was centrifuged at 14 000 rpm for 20 min at 4°C. The supernatant, as an enzyme extract, was kindly separated and used for determination of enzyme activity. Catalase (CAT) activity was determined based on the rate of disappearing H_2O_2 at 240 nm using a spectrophotometer, as described by Dhindsa [1981]. For measuring peroxidase (POD), 30 μL of 0.6% H_2O_2 and 130 μL of enzyme extract were added to 1.84 mL of reaction solution. Changes occurred in the light absorption were recorded at 465 nm, indicating a reduction in H_2O_2 concentration, this process was performed at a ten-second interval for 120 s [Plewa 1991]. Each unit of POD was considered as the content of enzyme responsible for a 1 μ mole reduction in H_2O_2 concentration for 1 min.

Fatty acid composition. In order to identify and quantify the composition of fatty acids in the oil samples through gas chromatography (GC), the olive oil in each sample was initially extracted based on Brito et al. [2018b] method. 1 μL of samples was taken and injected into a gas chromatography (Varian) equipped with a flame ionization detector (FID) and capillary column SI-88 in 60 m length. The temperature of injection port was 156°C and hydrogen was used as a carrier gas. Temperature program was 150°C/5 min and 175°C/35 min. The identification fatty acids in the samples were performed by comparing FAMEs retention times with those of reference standards. The results of FAs were expressed as percentages of total FAMEs.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). Significant differences between values were determined by Duncan's range test with differences being considered significant at $P < 0.05$. All analyses were performed with SAS software, version 9.1.

RESULTS

Fruits yield. The results showed that in the both cultivars, RDI with 50% ETc decreased fruit yield, as compared to full irrigation (control). In Zard cultivar, foliar spray at 100% and 75% ETc had no significant effect on fruit yield, whereas all sprayings (SA, KL, and SA + KL) at 50% ETc improved fruit yield as compared to controls. In 'Roghani', all sprayings, except for SA, enhanced fruit yield at full irrigation (100% ETC) as compared to the controls (Fig. 1).

Fruit weight. The results of this research showed that all foliar spray treatments improved fruit weight, as compared to the controls. The highest fruit weight was observed at 100% ETc. In the both cultivars, an

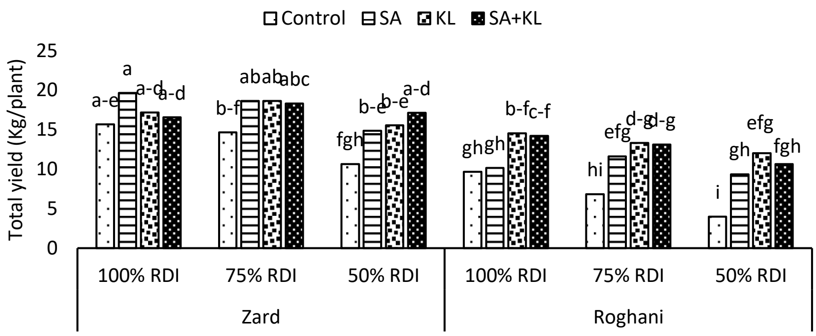

Fig. 1. Effects of foliar application of salicylic acid (SA) and kaolin (KL) on the total yield in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncanôs Multiple Range Test at P < 0.05

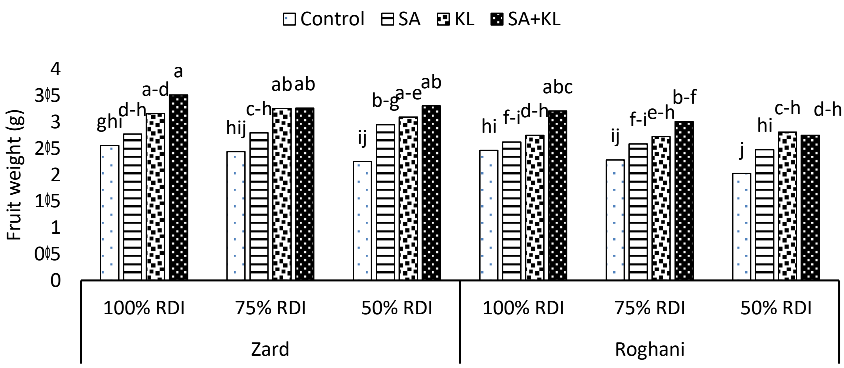

Fig. 2. Effects of foliar application of salicylic acid (SA) and kaolin (KL) on the fruit weight in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncanôs Multiple Range Test at P < 0.05

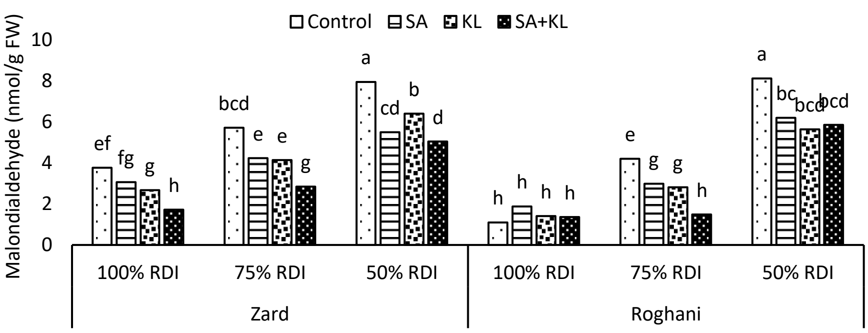

Fig. 3. Effects of foliar application of salicylic acid (SA) and kaolin (KL) solution on the malondialdehyde content in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncanôs Multiple Range Test at P < 0.05

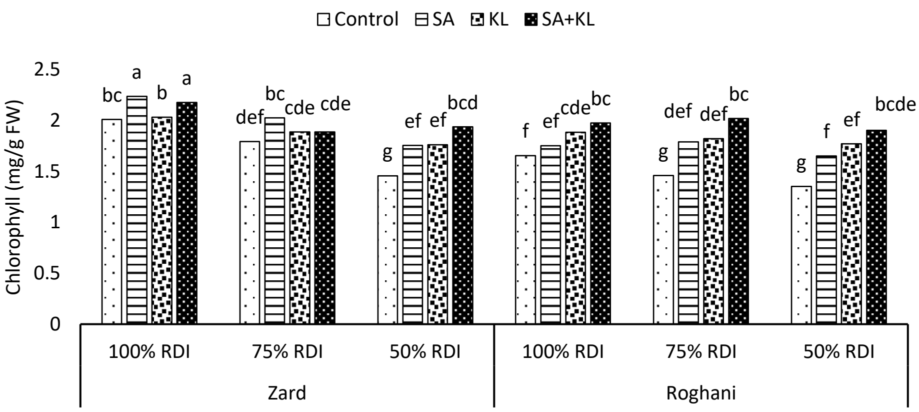

Fig. 4. Effects of foliar application of salicylic acid (SA) and kaolin (KL) solution on the chlorophyll content in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncan's Multiple Range Test at $P < 0.05$

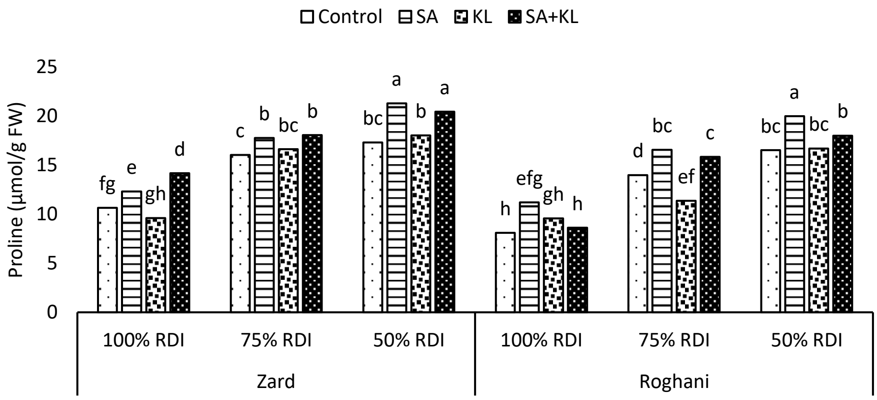

Fig. 5. Effects of foliar application of salicylic acid (SA) and kaolin (KL) on the proline in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncan's Multiple Range Test at $P < 0.05$

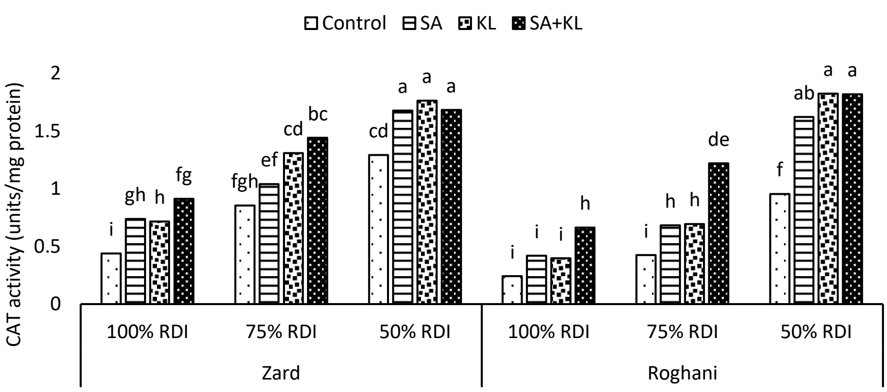

Fig. 6. Effects of foliar application of salicylic acid (SA) and kaolin (KL) on the CAT activity in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncan's Multiple Range Test at $P < 0.05$

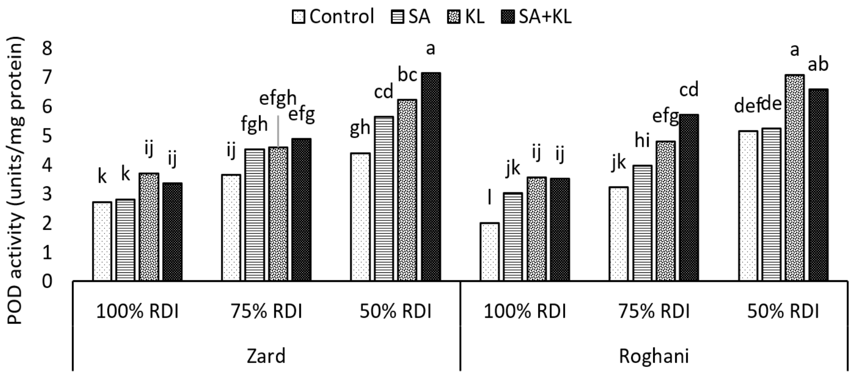

Fig. 7. Effects of foliar application of salicylic acid (SA) and kaolin (KL) solution on the POD activity in two cultivars of olive (Zard and Roghani) subjected to regulated deficit irrigation (RDI). At each cultivar, values with the different letters are significantly different according to Duncan's Multiple Range Test at $P < 0.05$

increase of 25 to 29% fold in fruit weight was obtained by applying KL and SA + KL treatments (Fig. 2).

Chlorophyll content. The effect of treatments on chlorophyll content in the both cultivars was presented in Figure 3. In the both olive cultivars, the content of chlorophyll was various under an interaction of irrigation × spraying. In the both cultivars, applying SA or KL under 50% ETc improved chlorophyll content as compared to the controls. In particular, an interaction effect of SA + KL gained the highest chlorophyll content rather than the other treatments (Fig. 4).

Malondialdehyde. In the both cultivars, the accumulation of MDA significantly amplified with decreasing irrigation. In Zard cultivar, spraying SA and KL solutions, either separately or simultaneously, significantly decreased MDA accumulation at all irrigations. In Roghani, the both solutions depressed MDA content at 50% and 75% ETc, as compared to the controls. However, the highest MDA suppression was attained by a SA + KL treatment (Fig. 3).

Proline. In the both cultivars, proline was significantly increased, as level of irrigation decreased. Interestingly, SA and KL had a different effect on proline content. For example, SA significantly increased proline at 50% ETc, as compared to the controls. In Roghani, KL solution contrarily had no significant effect on proline accumulation at different levels of irrigation, except for 75% ETc, as compared to the controls. Also, applying SA + KL in Zard cultivar led to an increase in proline accumulation, as compared to the controls (Fig. 5).

Catalase activity. In the both cultivars, the CAT activity was elevated with decreasing irrigation level. In this context, spraying SA and KL solutions on leaves of both cultivars significantly enhanced CAT activity, as compared to the controls. Moreover, the highest CAT activity was obtained by an interaction of SA+ KL solutions (Fig. 6).

Peroxidase activity. Effect of the treatments on activity of POD was shown in Fig. 9. In the both cultivars, low irrigations increased peroxidase activity. Spraying SA and KL solutions on leaves of the both cultivars significantly increased peroxidase activity, as compared to the controls. However, no significant difference was found between KL and SA + KL treatments at full irrigation (100% ETc) in the both olive cultivars (Fig. 7).

Fatty acid composition. The results showed that both interactions of irrigation × cultivar and irrigation × sprayings had no significant effect on rate of palmitic acid, oleic acid, and saturated and unsaturated fatty acids in fruits. In Roghani cultivar, the rate of palmitoleic acid, linoleic acid, and linolenic acid was higher than those in Zard cultivar. On the contrary, rate of unsaturated/saturated fatty acids (UFA/SFA) in Zard cultivar was higher rather than those in Roghani cultivar. Furthermore, applying different irrigation regimes and foliar sprayings had different impacts on percentage

Table 2. Fatty acids composition of Zard and Roghani olives cultivars as affected by regulated deficit irrigation (RDI) and foliar salicylic acid (SA) and kaolin (KL) treatments

Parameter	ANOVA	Cultivar	RDI1				RDI2				RDI3			
			control	SA	KL	SA + KL	control	SA	KL	SA + KL	control	SA	KL	SA + KL
Palmitic	ns	Zard	12.47	11.46	11.61	10.93	13.43	12.51	12.72	11.60	13.66	13.59	13.67	12.24
		Roghani	17.25	14.39	15.55	12.53	18.03	15.69	16.86	13.75	18.35	17.51	18.22	14.55
Palmitoleic	***	Zard	0.86k	0.67mn	0.78klm	0.61n	1.18hi	0.67mn	0.85kl	0.73lmn	1.33hij	1.02j	1.16hi	0.66mn
		Roghani	1.97a	1.49de	1.17hi	1.1ij	1.74bc	1.24gh	1.34fg	1.20hi	1.85b	1.69c	1.56d	1.41ef
Stearic	***	Zard	3.42ghi	2.27kl	2.71j	2.06l	4.23abc	2.58jk	3.65efgh	2.24kl	4.39ab	4.05bcde	4.59a	2.54jk
		Roghani	3.24hi	3.67efg	3.93cdef	3.23i	3.56fghi	4.08bcd	4.37ab	3.56fghi	3.76defg	3.52fghi	3.45ghi	3.70defg
Oleic	ns	Zard	64.73	69.20	70.66	70.34	63.71	66.19	64.88	69.43	62.62	66.22	63.42	66.30
		Roghani	56.60	58.65	57.95	63.55	56.01	57.64	58.56	62.24	54.43	56.62	55.25	60.16
Linoleic	***	Zard	17.29def	15.71ijk	16.64efgh	14.56l	16.26ghi	16.91d-h	16.98d-g	15.50jk	16.53fgh	15.16kl	16.15hij	17.07def
		Roghani	18.80bc	18.48bc	19.06abc	18.39c	18.96abc	19.08abc	17.51d	17.36de	19.62a	18.35c	19.25ab	18.58bc
Linolenic	***	Zard	0.63gh	0.57h	0.65fgh	0.72fg	0.73efg	0.63fg	0.63fg	0.74efg	0.84e	0.68fgh	0.76ef	0.64fgh
		Roghani	1.06cd	1.22b	1.21b	1.64a	1.07cd	1.22b	1.15bc	1.04cd	1.11bc	0.96d	1.15bc	1.16bc
Arachidic	***	Zard	0.22jk	0.24ij	0.19k	0.34b-e	0.30e-h	0.25ij	0.26hij	0.32c-f	0.35bcd	0.24ij	0.33cde	0.35bcd
		Roghani	0.25ij	0.28f-i	0.34b-e	0.33cde	0.36bc	0.22jk	0.31d-g	0.24ij	0.42a	0.34b-e	0.38ab	0.27ghi
SFA	ns	Zard	16.12	13.98	14.53	13.34	17.97	15.34	16.68	14.17	18.40	17.98	18.60	15.14
		Roghani	20.75	18.35	19.82	16.09	21.95	19.99	21.54	17.56	22.54	21.37	22.06	18.52
USFA	ns	Zard	83.53	86.16	88.75	86.24	81.89	84.41	83.35	86.41	81.14	83.09	81.50	84.68
		Roghani	78.44	79.85	79.39	84.68	77.79	79.19	78.56	81.86	77.01	77.64	77.22	81.32
SFA/USFA	*	Zard	5.18bc	6.16a	6.12a	6.47b	4.56e	5.50b	5.01cd	6.10a	4.41ef	4.64de	4.38ef	5.59b
		Roghani	3.78hij	4.36efg	4.00fgh	5.26bc	3.54ij	3.96ghi	3.65hij	4.67de	3.41j	3.63hij	3.50j	4.40ef

SFA: saturated fatty acids, USFA: unsaturated fatty acids. For each trait, means followed by different letters are significantly different at $P \leq 0.05$ level by Duncan's test.

of fatty acids in the both cultivars. In general, as level of irrigation was diminished, the rate of palmitoleic acid, stearic acid, and arachidonic acid in oil raised, but rate of linoleic acid and linolenic acid remained constant. As compared to full irrigation (100% ETc), 75 and 50% ETC significantly reduced UFA/SFA in both cultivars, especially in Zard cultivar. Also, the results of our research showed that spraying SA and KL solutions under different irrigations had different effects on rate of fatty acids in the cultivars. In this context, SA and KL, either separately or simultaneously, significantly reduced the content of palmitoleic acid and stearic acid in Zard cultivar, as compared to the controls. However, in the both cultivars, a treatment of SA + KL increased UFA/SFA. In addition, application of SA and KL in Zard cultivar, increased UFA/SFA at 75% and 100% ETc (Tab. 2).

DISCUSSION

Olive is a well-known tolerant plant to drought, and this contributes to its physiological and biochemical responses, such stomatal conductance, evapotranspiration, antioxidant systems, and other mechanism, towards drought [Gholami and Zahedi 2019a]. However, a response to a given stress is dependent on type of species; for this reason, responses to water deficit between olive cultivars are various [Gholami and Zahedi 2019b, Petridis et al. 2012]. Broadly speaking, under drought and other environmental stresses, an increase in accumulation of reactive oxygen species (ROS) equals to incidence of oxidative damages [Doupis et al. 2013]. By measuring the rate of peroxidation of membrane lipids, the magnitude of damage exerted to the plants is estimated [Ghanbari and Sayyari 2018]. ROS negatively influences chlorophyll biosynthesis; as a result, it is able to reduce the yield of crop during water deficit [Yaser et al. 2008]. In the present study, low irrigation notably provided an opportunity to emerge MDA accumulation, and chlorophyll collapse in the both olive cultivars, as compared to the controls. For example, 50% ETc significantly reduced yield in the both olive cultivars, compared to full irrigation. In a similar way, it was also reported that applying RDI significantly decreased olives total yield, as compared to the controls [Gholami and Zahedi 2019b, Talozi and Al Waked 2016].

The results showed that spraying SA and KL on olive trees led to a reduction in accumulation of MDA, while improved chlorophyll and total yield, as compared to the controls. Under different irrigation regimes, a reduction in oxidative damage and an increase in yield of the cultivars are probably contributed to some defensive mechanism triggered by exogenous application of SA and KL [Noreen and Ashraf 2010, Khaleghi et al. 2015]. As a plant-derived phenol, SA regulates plants' response to stressful conditions. Under stressful condition, this hormone improves antioxidant systems and enhances production of some plant hormone like ABA [Brito et al. 2019a]. Similarly, KL improves antioxidant systems and enhances relative moisture in stressed plants; and this induces plants' tolerance to drought [Glenn 2012].

In the present study, applying SA and KL significantly increased not only CAT and POD activity, but also chlorophyll content and proline accumulation. On the contrary, they decreased accumulation of MDA in favor of improving yield of treated plants compared to the controls. In this respect, applying SA and KL separately altered plants' hormones and improved antioxidant capacity in order to alleviate harmful effect of stresses on the plants and enhance their yield, as compared to the controls [Brito et al. 2018b, Brito et al. 2019a]. In our research, the efficiency of SA and KL on drought was examined and the results revealed that an interaction treatment of SA and KL ameliorated most of traits investigated here. This result may be due to their role on inducing plants' tolerance to the stressful condition.

The quality of plant-derived oils is contributed to the percentage of UFA in their composition [Lunn and Theobald 2006]. In this study, the average of saturated fatty acids and unsaturated fatty acids in the extracted oil was 13–27% and 77–88%, respectively, indicating high quality of olive oil under the predetermined treatments. As cited above, the olive oil in this research was extracted from the both cultivars subjected to water deficit. This stress enhanced its palmitoleic acid, stearic acid, and linoleic acid, but had no significant effect on the other fatty acids. In general, although the status of fatty acids in olive oil at different irrigations has not been affected [Patumi et al. 2002], low irrigation was reported to implement some changes in its fatty acids composition [Cano-Lamadrid et al. 2015]. However, the results of this research showed that RDI reduced the ratio of UFA/SFA in the olive oil. It is noteworthy that UFA are useful for human health [Lunn and Theobald 2006]. In a study, a reduction in UFA/SFA was found in Manzanilla cultivar of olive under low irrigation [Cano-Lamadrid et al. 2015]. A reduction in quality of olive oil due to low irrigation was also reported in other studies [Dabbou et al. 2015, Dag et al. 2015]. Based on the results of these studies, it seems that water deficit has a harmful effect on quality of olive oil. Therefore, SA and KL may mitigate deleterious effect of drought on oil of the stressed plants. In our study, SA and KL also increased ratio of UFA/SFA and thereby enhancing the quality of olive oil. It seems that they, through depressing harmful effects of drought on the plants, retain the qualitative characteristics of olive oil. In this regard, KL has been reported to reduce saturated fatty acids and increase MUFAs content in olive oil, as compared to the controls [Khaleghi et al. 2015]. In addition, SA significantly increased rate of linolenic in the lines of sunflowers under saline stress [Noreen and Ashraf 2010]. Therefore, KL and SA partly may alleviate the harmful effect of drought stresses influencing quality of olive oil.

In general, the results of this research revealed that employing kaolin and SA (particularly in combination) improved olive tolerance towards drought stress and consequently enhanced its yield under this condition. Through ameliorating antioxidant system and maintaining chlorophyll content of the stressed olive plants, the external application of kaolin and SA significantly reduced malondialdehyde accumulation. Furthermore, a combination of kaolin and SA improved ratio of UFA/SFA and enhanced the quality of oil extracted from them under drought stress. Therefore, kaolin and SA are recommended for alleviating the adverse effects of drought stress on olive plants and maintaining their yield and fruit quality under this condition.

SOURCE OF FUNDING

Authors' private funds.

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