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Effect of foliar application of amino acid, humic acid and fulvic acid on the oil content and quality of olive

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ABSTRACT

Olive is an evergreen tree with a low growth rate that is cultivated in semi-tropical climates. The biochemical properties of three olive cultivars were explored under the foliar application of amino and organic acids in a two-factor factorial experiment based on a randomized complete block design with three replications in Rudbar County, Iran. The first factor was assigned to olive cultivar ('Zard', 'Arbequina', and 'Manzanilla') and the second factor to the foliar application of organic acids at 9 levels of control, arginine, glutamine, humic acid, fulvic acid, arginine + humic acid, arginine + fulvic acid, glutamine + humic acid, and glutamine + fulvic acid. The recorded traits included the Brix value, content of oil, protein, chlorophyll, carotenoid, anthocyanins, phenols, and the activity of antioxidant enzymes. The Results showed that cv. 'Zard' had the highest Brix value, fruit protein content, carotenoid, anthocyanins, and phenols, and cv. 'Arbequina' had the highest oil fraction. The Results of the simple effects of organic acids revealed that the trees treated with arginine + humic acid had the highest fruit protein content and total chlorophyll, and those treated with humic acid had the highest anthocyanin and phenol contents. Data on the interaction of 'cultivar × organic acids' showed that 'Arbequina × glutamine' had the highest oil content, 'Manzanilla × fulvic acid' and 'Manzanilla × glutamine + fulvic acid' had the highest fruit protein content, 'Zard × humic acid' had the highest phenol content, 'Arbequina × arginine' had the highest superoxide dismutase activity, and 'Arbequina × glutamine + fulvic acid' had the highest peroxidase activity. Finally, it can be concluded that Arbequina cultivar produced the most oil when foliar sprayed with glutamine.

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1. Introduction

Olive (*Olea europaea* L.) is an evergreen tree with a slow growth rate that is cultivated in subtropical climates between latitudes 30° and 45° and is an important crop in most Mediterranean countries (Hagidimitriou and Pontikis, 2004). The overuse of chemical fertilizers in agriculture caused to various environmental problems, such as the physical degradation of soil and its nutrient imbalance (Aghili et al., 2009). So, the foliar application of nutrients has today turned into a common practice in most orchards with indisputable

optimal impacts on vegetative traits, yield, and fruit quality. During fruit growth and development in which competition between reproductive parts and roots on nutrient uptake reduces the activity of the roots, nutrient uptake is decreased too. This sort of competition can be decreased by the timely spray of nutrients (Andrade et al., 2009). Amino acids have a proven role as a biostimulator in plants exposed to biotic and abiotic stresses. These acids are involved in increasing the total protein content of the plants. The increased capacity of total proteins may be related to the increased concentration of ribosomal proteins and their role as the structural unit of proteins. Furthermore, plant cells can directly uptake amino acids as organic protein compounds and as the building block of proteins (Abd El-Aziz et al., 2009). Amino acids influence plant yields and growth by improving their tolerance to environmental stresses and increasing their chlorophyll and protein contents. As an osmotic factor in the cytoplasm of stomatal cells, glutamic acid affects stomatal opening and closure. Arginine, also, increases the synthesis of plant hormones that influence flowering and fruit-bearing (Pouryousef Miandoab and

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Shahravan, 2014). Amino acids are directly and indirectly influential on the physiological activities and the growth and development of plants (Faten et al., 2010).

The application of compounds containing free amino acids was effective in increasing the protein content of peas by increasing their nitrogen content. This study showed that the N, P, and K contents of peas were significantly increased with the foliar application of amino acids (Mahmoudi, 2012). The application of humic acid + chelated Fe increased Fe uptake in citrus and improved fruit yield and quality (Cerdán et al., 2007). Also, the application of 32–48 g humic acid to the pomegranates improved their vegetative growth, yield, and fruit quality (Khatab et al., 2012). Haghparast et al. (2011) stated that the application of 50 mg/L humic acid and fulvic acid increased the stem and root length of pepper seedlings. Apricots treated with an organic acid exhibited a higher level of TSS and a lower level of titratable acid, which naturally improved their fruit taste index (Fathy et al., 2010). A study on the application of humic acid to the pomegranates reported that total phenol content was decreased and total flavonoid content was increased over the growth period while the fruits were maturing (Kulkarni and Aradhya, 2005). The application of humic acid on grape cv. 'Askari' increased their cluster weight, berry weight, and biochemical parameters such as TSS and acidity (Mohamadineia et al., 2015). The treatment of Guava paluma with humic acid increased vitamin C synthesis and content, TSS, and titratable acidity (Rocha et al., 2016). Also, the foliar application of humic acid to apricots enhanced the biochemical parameters of their fruits (Shaaban et al., 2015). There are numerous reports as to the positive effect of humic acid on some horticultural crops such as olive (Hagagg et al., 2012), apricots (Fathy et al., 2010), and tomatoes (Dogan and Demir, 2014). So, the effects of two amino acids (arginine and glutamine) and two organic acids (humic and fulvic acids) were explored on three olive cultivars (Zard, Arbequina, and Manzanilla) in the present experiment in an olive orchard in the Aliabad region of Rudbar, Iran.

2. Materials and methods

2.1. Experimental design and treatments

The research was carried out as a two-factor factorial experiment based on a randomized complete block design with three replications (one tree per plot) and a total of 81 trees in the olive

commercial orchard located in Aliabad region of Rudbar County, Iran. Aliabad is located at latitude 36.746258 N. and longitude 49.282738 E. and has saline soil and water. The first factor was assigned to three olive cultivars (Zard, Arbequina, and Manzanilla) and the second factor to organic acids at nine levels including control, arginine (100 mg/L), glutamine (100 mg/L), humic acid (100 mg/L), fulvic acid (100 mg/L), arginine + humic acid (100 mg/L + 100 mg/L), arginine + fulvic acid (100 mg/L + 100 mg/L), glutamine + humic acid (100 mg/L + 100 mg/L), and glutamine + fulvic acid (100 mg/L + 100 mg/L). The foliar application of organic acids was conducted in two stages in May and August of 2019. The recorded traits included Brix value as degrees Brix, oil content, leaf and fruit protein contents, chlorophyll a, b, and total, carotenoids, anthocyanins, phenols, superoxide dismutase, catalase, peroxidase, and antioxidant capacity.

2.2. Brix index

The Brix value was measured with a handheld N-1 α refractometer (ATAGO, Japan). The fruit oil was extracted with a Soxhlet extractor using hexane solution and was calculated in percent of dry and fresh matter. The standard protein solution was prepared by dissolving 1 mg of BSA powder in 5 mL of double distilled water. Then, 5 mL of the Bradford solution and predetermined volumes of the standard BSA solution (20–200 μ L) were poured into test tubes where they were adjusted to 500 μ L by adding distilled water. Then, the absorbance of the standard chromatic solutions was individually read at 595 nm with a Shimadzu 160-UV spectrophotometer (Asgari et al., 2012).

2.3. Chlorophyll content

To measure chlorophyll content, 0.5 g of the sample was crushed in 50 mL of 80% acetone (80 mL of acetone + 20 mL of distilled water). Then, the extract was filtered, adjusted to 50 mL, and poured into cuvettes. The chlorophyll content was determined by a spectrophotometer. It was read at 643 and 660 nm. Then, the readings were placed in the following equations to find out chlorophyll a, chlorophyll b, and total chlorophyll contents (Mazumdar and Majumder, 2003).

$$\text{Total chlorophyll (mg/mL)} = 7.12 (A_{660}) + 16.8 (A_{643})$$

$$\text{Chlorophyll a (mg/mL)} = 9.93 (A_{660}) - 0.777 (A_{643})$$

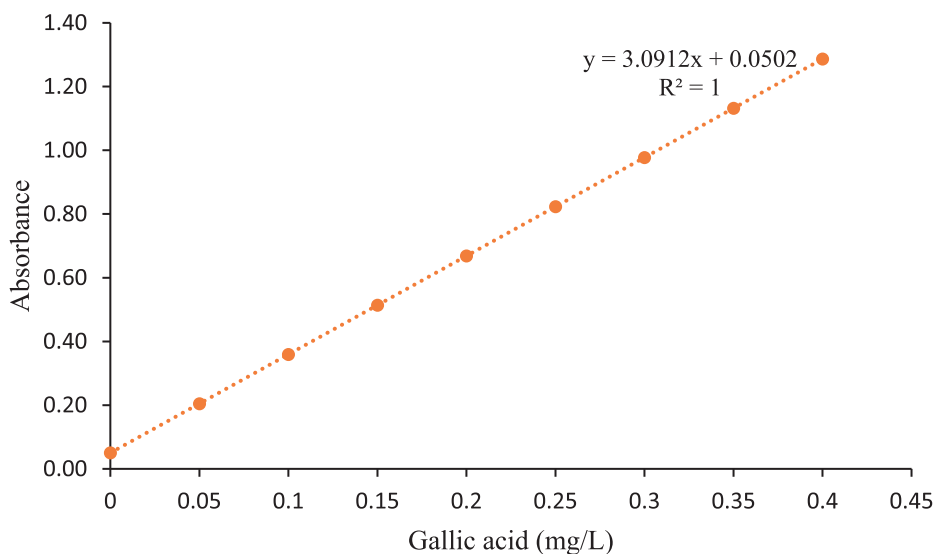


Fig. 1. The standard curve of Gallic acid.

Chlorophyll *b* (mg/mL) = 17.6 (A_{643}) – 2.81 (A_{660})

2.4. Carotenoid and anthocyanin

To determine carotenoid content, the treatments were sampled. So, 0.5 g of the sample was weighed and crushed in a china mortar containing 50 mL of 80% acetone (80 mL of acetone + 20 mL of distilled water). Then, the extract was filtered, adjusted to 50 mL, and poured into cuvettes. The extracts were read at three wavelengths of 645, 663, and 660 nm, and the following equations were employed to find out the carotenoid contents of the treatments (Mazumdar and Majumder, 2003).

Carotenoid content = 4.69 (A_{660}) – 0.268 (A_{645}) + 8.02 (A_{663})

To estimate anthocyanin content, 0.5 g was taken from each sample and crushed in a china mortar with 50 mL of ethanol-hydrochloric acid (85% of ethanol 85% + 15% of hydrochloric acid). The extract was then filtered, adjusted to 50 mL, and poured into cuvettes. The cuvettes were refrigerated at 4 °C for 24 h followed by keeping in darkness for 2 h. Then, they were read at 535 with a spectrophotometer and the following equation was used to estimate the anthocyanin content of the treatments (Mazumdar and Majumder, 2003).

Sample weight (0.5g) = $\frac{e \times b \times c}{d \times a} \times 100$

in which *e* is the reading at 535 nm, *b* is the volume taken for sampling (5 mL), *c* is the total volume (50 mL), *d* is the fraction taken for sample (0.1 mL), and *a* is the sample weight (0.5 g).

Total anthocyanin content of a sample

= $\frac{\text{Total absorbance of the sample}}{98.2}$

2.5. Phenolic compounds

The total polyphenol content was determined by the Folin-Ciocalteu reagent (FCR). To this end, about 1 g of fresh leaves was ground in 10 mL of methanol for 2 min. The resulting solution was subsequently filtered. Then, 0.5 mL of diluted extract (1:10 g/mL) was added with 5 mL of diluted FCR (1:10 diluted with distilled water) and then 4 mL of sodium carbonate solution (7.5% v/v). The samples were then kept at laboratory temperature for 15 min after which their absorbance was read at 765 with a spectrophotometer (McDonald et al., 2001). The standard graph was developed by using different concentrations of gallic acid (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, and 0.4 mg/mL), and the total phenol content was calculated for each sample by the standard curve and the resulting line equation (Fig. 1).

2.6. Antioxidant enzymes and capacity

The activity of the superoxide dismutase (SOD) was estimated by Das et al's (2000) procedure. So, 1.4 mL of the reaction compound was added to 100 μ L of the sample extract and was kept at 30 °C for 5 min. Then, 80 μ L of 50 μ M riboflavin was added to it and it was exposed in test tubes to 200-W Philips fluorescent lamps for 10 min. Then, 1 mL of Griess reagent (a mixture of 1% sulfanilamide and 5% phosphoric acid in equal proportions) was added, and the absorbance was read at 543 nm. Each unit of SOD activity was considered to be an amount of SOD with a 50% inhibitory effect on nitrite formation under the measurement conditions.

To measure the catalase (CAT) activity, 0.01 M of phosphate buffer (pH 7), 0.5 mL of 0.2 M H₂O₂, and 2 mL of an acidic reagent (a dichromate/acetic acid mixture) was first added to 1 g of plant

tissue already crushed in 4 mL of ethanol. Then, absorbance was read at 610 nm with a spectrophotometer (Chance and Maehly, 1995). To measure the activity of peroxidase (POD), the relevant extract was prepared and the OD variations were read at 430 nm every 30 s for 2 min with a spectrophotometer (Chance and Maehly, 1995).

To determine antioxidant capacity, 1 g of the plant was placed in liquid nitrogen for 2–3 min. Then, it was ground with 10 mL of 85% methanol, and the samples were filtered and centrifuged for 5 min. Of this amount, 150 mL was taken and added with 850 μ L of DPPH. Then, the samples were read at 517 nm with a spectrophotometer. The antioxidant capacity of the extracts was calculated as percent DPPH inhibition using the following equations (Ramandeep and Savage, 2005).

%DPPH_{sc} = $\frac{A_{cont} - A_{samp}}{A_{cont}} \times 100$

in which %DPPH_{sc} represents the percent inhibition, A_{cont} represents the amount of DPPH absorption, and A_{samp} represents the amount of absorption (DPPH + sample). Data were statistically analyzed in the MSTATC software package, and the means were compared by the LSD test.

3. Results

3.1. Brix value

The analysis of variance showed that the simple effect of cultivar was significant ($P \leq 0.01$) on the Brix value, but this trait was not significantly affected by the simple effect of organic acids and the interactive effect of 'cultivar \times organic acids' (Table 1). The comparison of the means showed that cv. 'Zard' had the highest and cv. 'Arbequina' had the lowest Brix value (Fig. 2). These two cultivars showed an about 38% difference in the Brix value, which is of importance qualitatively.

3.2. Oil content

The oil content was significantly affected by the simple effect of cultivar ($P \leq 0.01$) and the interaction of 'cultivar \times organic acids' ($P \leq 0.05$), but organic acids could not change this trait significantly (Table 1). As the comparison of the means for the effect of the cultivar (Fig. 2) showed, the highest oil content was obtained from 'Arbequina' (51.014%) and the lowest from 'Zard' (43.57%) and 'Manzanilla' (40.18%). These two latter cultivars did not differ significantly in this trait. The comparison of the means for the interactive effect of 'cultivar \times organic acids' (Table 4) revealed that 'Arbequina \times glutamine' produced the highest oil content and 'Manzanilla \times arginine + fulvic acid' produced the lowest one. This latter treatment differed in the oil content with the superior treatment by about 200%.

3.3. Leaf and fruit protein content

According to the analysis of variance, the simple effects of cultivars and organic acids were not significant on leaf protein. But, their interaction was significant ($P \leq 0.01$) for this trait (Table 1). Based on the comparison of means for the interactive effect of 'cultivar \times organic acids' on leaf protein content, the highest content was obtained from 'Arbequina \times control', although the leaf content of 'Arbequina' treated with either arginine, glutamine, human acid, or fulvic acid did not differ from the control (the superior treatment) significantly. The lowest leaf protein content was related to the interaction of 'Zard \times glutamine' (Table 4).

Table 1
The analysis of variance for the effect of the experimental factors on the traits of three olive cultivars, i.e., 'Zard', 'Arbequina', and 'Manzanilla'

Sources of variations	df	Means of squares													
		Brix	Oil	Leaf protein	Fruit protein	Chl.a	Chl.b	Total chl.	Carotenoid	Anthocyanin	Phenol	Superoxide dismutase	Catalase	Peroxidase	Antioxidant capacity
Replication	2	0.63 ^{ns}	76.98 ^{ns}	0.37 ^{**}	0.065 ^{**}	0.57 ^{**}	0.25 ^{ns}	1.37 ^{**}	3.09 ^{**}	23.96 ^{ns}	0.71 ^{**}	5.35 ^{**}	0.001 ^{ns}	0.004 ^{ns}	228.82 ^{**}
Cultivar (A)	2	233.09 ^{**}	850.49 ^{**}	0.03 ^{ns}	0.014 [*]	0.24 ^{ns}	0.43 ^{**}	0.10 ^{ns}	1.85 ^{**}	56.04 [*]	2.03 ^{**}	0.76 ^{ns}	0.004 ^{**}	0.074 ^{**}	21.56 ^{ns}
Organic acid (B)	8	2.78 ^{ns}	30.13 ^{ns}	0.03 ^{ns}	0.014 ^{**}	0.12 ^{ns}	0.28 ^{**}	0.53 [*]	0.04 ^{ns}	39.39 ^{**}	0.25 ^{ns}	11.63 ^{**}	0.001 ^{**}	0.051 ^{**}	47.06 ^{**}
AB	16	2.66 ^{ns}	88.85 [*]	0.05 ^{**}	0.010 ^{**}	0.23 [*]	0.19 [*]	0.27 ^{ns}	0.21 ^{ns}	35.04 ^{**}	0.29 [*]	2.47 ^{**}	0.001 ^{ns}	20.047 ^{**}	18.05 ^{ns}
Error	52	1.71	42.55	0.02	0.004	0.10	0.10	0.23	0.19	13.33	0.13	1.04	0.000	0.00	12.91
CV		10.37	14.51	14.40	14.07	28.98	26.47	21.06	23.12	25.61	18.95	41.93	38.58	30.93	5.20

ns: non-significant difference; **: significant difference at the P ≤ 0.01 level; *: significant difference at the P ≤ 0.05 level.

Based on the results (Table 1), fruit protein content was significantly affected by the simple effects of cultivar (P ≤ 0.05), the simple effects of organic acids (P ≤ 0.01), and the interactive effects of these two factors (P ≤ 0.01). It was found from the comparison of the means that the highest fruit protein content was related to 'Zard' and the lowest to 'Arbequina' (Fig. 2). Data for the effect of organic acids on this trait revealed that the treatment of glutamine was related to the highest and the treatment of arginine + humic acid was related to the lowest fruit protein content, which was not significantly different from that of the control and glutamine + fulvic acid (Table 3). The comparison of the means for the interactive effect of 'cultivar × organic acids' (Table 4) indicated that 'Manzanilla × fulvic acid' and 'Arbequina × glutamine' produced the highest fruit protein content, whereas 'Arbequina × arginine + humic acid' produced the lowest one.

3.4. Chlorophyll content

Analysis of variance indicated that the simple effect of cultivar and organic acids were not significant on chlorophyll a content, but their interaction influenced this trait significantly (Table 1). Based on the comparison of the means (Table 4), the highest chlorophyll a content was related to the treatment of 'Zard × arginine + humic acid' and the lowest to the treatment of 'Zard × glutamine + fulvic acid'. Chlorophyll b content was significantly influenced by both simple and interactive effects of cultivar and organic acids (Table 1). Data on organic acids revealed that humic acid was related to the lowest chlorophyll b content of 0.76 mg/mL (Table 3). Data on the interaction of 'cultivar × organic acids' (Table 4) showed that the highest chlorophyll b content was related to the interaction of 'Arbequina × arginine' and the lowest to the interaction of 'Zard × humic acid'. The simple effect of organic acids was significant (P ≤ 0.05) on total chlorophyll content, but the simple effect of olive cultivars and the interactive effect of 'cultivar × organic acids' were not significant. The comparison of the means indicated that the plants treated with 'arginine + humic acid', 'glutamine + humic acid', glutamine, or arginine produced the highest amount of chlorophyll, whereas those treated with humic acid produced the lowest one (Table 3).

3.5. Carotenoid content

Different olive cultivars significantly (P ≤ 0.01) influenced leaf carotenoid content, but the simple effect of organic acids and the interactive effect of 'cultivar × organic acids' were not significant on this trait (Table 1). As the comparison of the means showed, 'Zard' had the highest and 'Manzanilla' had the lowest carotenoid content (Table 2), so that their difference amounted to over 300%. In other words, carotenoid content in the leaves of 'Zard' was three times higher than carotenoid content in the leaves of 'Manzanilla'.

3.6. Anthocyanin content

As the analysis of variance showed, the simple and interactive effects of both factors, i.e., cultivar and organic acids, were significant on anthocyanin content (Table 1). The comparison of the means (Table 2) revealed that 'Zard' and 'Manzanilla' had the highest anthocyanin content and 'Arbequina' had the lowest. The comparison of the means for the effect of organic acids (Table 3) showed that the plants treated with humic acid exhibited the highest anthocyanin content of 17.79 mg/100 g. The comparison of the means for the interaction of 'cultivar × organic acids' showed that 'Manzanilla × control' and 'Manzanilla × arginine + humic acid'

Table 2
The comparison of the means for the effect of cultivar on the studied traits of three olive cultivars.

Cultivar	Chlorophyll <i>b</i> (mg/mL)	Carotenoid (mg/L)	Anthocyanin (mg/100 g)	Phenol (mg/100 g)	Catalase (UNIT)*	Peroxidase (UNIT)*
'Zard'	1.05b	7.12 a	15.44 a	2.10 a	0.037c	0.085c
'Arbequina'	1.30 a	5.65b	1.65b	2.05 a	0.062 a	0.188 a
'Manzanilla'	1.16 ab	2.34c	14.67 a	1.60b	0.047b	0.120b

Similar letter(s) in each column shows significant differences based on the LSD test.

* Catalase and peroxidase are expressed in $\mu\text{M H}_2\text{O}_2$ consumed/min/mg protein.

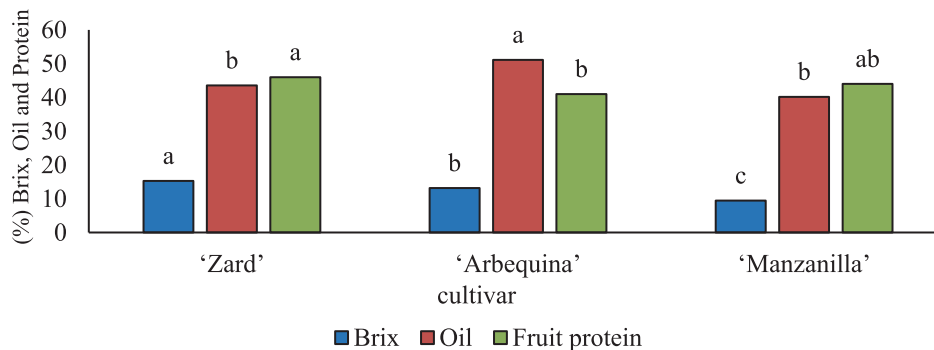


Fig. 2. The comparison of the means for the effect of cultivar on the Brix value and fruit oil and protein contents of three olive cultivars, i.e., 'Zard', 'Arbequina', and 'Manzanilla'

Table 3
The comparison of the means for the interactive effects of organic acids on the studied traits of three olive cultivars.

Organic acids	Fruit protein (%)	Chlorophyll <i>b</i> (mg/mL)	Total chlorophyll (mg/mL)	Anthocyanin (mg/100 g)	Superoxide dismutase (IU/g FW/min)	Catalase (UNIT)*	Peroxidase (UNIT)*	Antioxidant capacity (DPPH%)
Control	0.41 d	1.28 a	2.22 abc	17.75 ab	3.81 ab	0.047 cd	0.06c	70.08 ab
Arginine	0.42 cd	1.31 a	2.43 a	12.22c	4.30 a	0.060 ab	0.14b	71.97 a
Glutamine	0.50 a	1.26 a	2.48 a	14.29 bc	1.32 ef	0.034 e	0.17b	63.70c
Humic acid	0.48 ab	0.76b	1.87c	17.79 a	2.06 de	0.039 de	0.04c	68.72 ab
Fulvic acid	0.48 ab	1.10 a	2.14 abc	12.72c	1.05f	0.045 d	0.23 a	70.15 ab
Arginine + humic acid	0.39 d	1.24 a	2.53 a	13.10c	1.78 def	0.041 de	0.13b	69.15 ab
Arginine + fulvic acid	0.44 bcd	1.29 a	2.34 ab	14.14c	2.58 cd	0.045 d	0.07c	70.43 ab
Glutamine + humic acid	0.47 abc	1.22 a	2.38 a	13.13c	1.71 def	0.054 bc	0.07c	68.50b
Glutamine + fulvic acid	0.41 d	1.05 a	1.90 bc	13.15c	3.26 bc	0.070 a	0.25 a	69.41 ab

Similar letter(s) in each column shows significant differences based on the LSD test.

* Catalase and peroxidase are expressed in $\mu\text{M H}_2\text{O}_2$ consumed/min/mg protein.

had the highest and lowest anthocyanin contents, respectively (Table 4).

3.7. Polyphenol content of fruit

The analysis of variance revealed the significant effect of cultivar and 'cultivar × organic acids' on the fruit phenolic index. But, the simple effect of organic acids was not significant on this trait (Table 1). Among the cultivars, 'Zard' and 'Arbequina' had the highest and 'Manzanilla' had the lowest polyphenol content (Table 2). Among the interactions too (Table 4), the highest polyphenol content was related to 'Zard × humic acid' and the lowest to 'Zard × fulvic acid'.

3.8. Antioxidant enzymes and capacity

The analysis of variance for the effect of the experimental factors on SOD activity (Table 1) revealed that the simple effects of organic acids and the interactive effects of 'cultivar × organic acids' were significant ($P \leq 0.01$) on this trait, but the simple effect of cultivar was not significant. The highest SOD activity was related to

the plants treated with arginine and the lowest to those treated with fulvic acid (Table 3). Based on the comparison of the means for the interactive effects of 'cultivar × organic acids' (Table 4), 'Arbequina × arginine' exhibited the highest SOD activity and 'Manzanilla × fulvic acid' exhibited the lowest so that their difference in SOD activity was over 5.9 times.

According to the analysis of variance (Table 1), CAT was significantly ($P \leq 0.01$) affected by the simple effects of cultivars and organic acids, but their interaction was not significant. The comparison of the means revealed that the highest CAT was obtained from 'Arbequina' and the lowest from 'Zard' (Table 2). Among the organic acid treatments, glutamine + fulvic acid and glutamine alone exhibited the highest and lowest CAT, respectively (Table 3).

It was found by the analysis of variance that the simple and interactive effects of cultivar and organic acids were significant ($P \leq 0.01$) on the POD enzyme (Table 1). The comparison of the means for the effects of cultivar showed that the highest POD activity was exhibited by 'Arbequina' and the lowest by 'Zard' (Table 2). The comparison of the means for the effects of organic acids indicated that the plants treated with glutamine + fulvic acid and those treated with fulvic acid had the highest and the control

Table 4

The comparison of the means for the interactive effect of 'cultivar × organic acids' on the traits of three olive cultivars.

Cultivar	Organic acid	Oil (%)	Leaf protein (%)	Fruit protein (%)	Chlorophyll a (mg/mL)	Chlorophyll b (mg/mL)	Anthocyanin (mg/100 g)	Phenol (mg/100 g)	Superoxide dismutase (IU/g FW/min)	Peroxidase (UNIT)*
'Zard'	Control	39.46e-h	0.91b-f	0.43b-e	0.92cde	1.19b-g	15.92cde	2.54ab	4.92ab	0.04gh
	Arginine	45.72b-g	0.97b-f	0.48a-d	1.36abc	0.78f-i	13.51d-g	1.20b-g	2.18d-h	0.17c-g
	Glutamine	39.07fgh	0.66 g	0.47a-e	1.37abc	1.36a-e	19.68abc	1.69e-h	1.92e-h	0.22b-e
	Humic acid	41.11e-h	0.81efg	0.52ab	1.33abc	0.59i	16.69bcd	2.85a	2.01d-h	0.05gh
	Fulvic acid	52.57a-d	0.94b-f	0.41cde	0.94cde	1.20b-g	11.37d-g	1.38 h	1.19gh	0.11d-h
	Arginine + humic acid	45.47b-g	1.13ab	0.45b-e	1.85a	0.88e-i	15.67cde	2.34abc	1.37fgh	0.04gh
	Arginine + fulvic acid	46.67b-f	1.13ab	0.46a-e	1.00cde	1.23a-g	15.50c-f	2.19b-e	2.04d-h	0.02 h
	Glutamine + humic acid	41.90d-h	0.88c-g	0.51abc	1.59ab	1.22a-g	14.31c-g	1.71d-h	1.85e-h	0.04gh
	Glutamine + fulvic acid	40.18e-h	1.03a-e	0.44b-e	0.57e	0.98d-i	16.32 cd	2.21b-e	2.79c-g	0.07fgh
	'Arbequina'	Control	55.37ab	1.22a	0.37ef	1.05cde	1.11b-h	13.37def	1.89c-h	2.37d-h
Arginine		45.60b-g	1.03a-e	0.30f	1.08b-e	1.71a	9.56 fg	2.02b-g	6.27a	0.02 h
Glutamine		62.67a	1.03a-e	0.56a	1.19bcd	1.33a-e	11.26d-g	1.93c-h	0.84 h	0.05gh
Humic acid		53.64abc	1.07a-d	0.46a-e	0.86cde	0.95d-i	14.03c-g	2.11b-e	1.87e-h	0.04gh
Fulvic acid		47.97b-f	1.00a-e	0.47a-e	0.90cde	1.49abc	15.93cde	1.77c-h	0.89 h	0.33ab
Arginine + humic acid		48.75b-f	0.84d-g	0.29f	0.85cde	1.42a-d	14.13c-f	2.34abc	1.97d-h	0.19c-f
Arginine + fulvic acid		49.07b-f	0.88c-g	0.45b-e	1.05cde	1.28a-f	13.47d-g	2.05b-f	2.87c-f	0.14d-h
Glutamine + humic acid		47.19b-f	0.94b-f	0.45b-e	1.15bcd	1.26a-f	9.97efg	2.06b-f	1.66fgh	0.08e-h
Glutamine + fulvic acid		50.04b-e	1.10abc	0.41cde	1.20bcd	1.15b-g	12.16d-g	2.31a-d	3.38b-e	0.42a
'Manzanilla'		Control	35.34gh	0.94b-f	0.42b-e	1.04cde	1.56ab	23.94a	1.47fgh	4.14bc
	Arginine	40.67e-h	0.75 fg	0.47a-e	1.04cde	1.46a-d	13.58d-g	1.43gh	4.45bc	0.29abc
	Glutamine	39.77e-h	0.97b-f	0.47a-e	1.11bcd	1.09b-i	11.95d-g	1.95b-h	1.21 fgh	0.24bcd
	Humic acid	41.22e-h	0.94b-f	0.46a-e	1.21bcd	0.73ghi	22.65ab	1.70e-h	2.30d-h	0.04gh
	Fulvic acid	40.50 e-h	1.03a-e	0.56a	1.27bcd	0.62hi	10.85d-g	1.79c-h	1.06 h	0.11d-h
	Arginine + humic acid	47.77b-f	1.03a-e	0.43b-e	1.15bcd	1.42a-d	9.50 g	1.51fgh	2.01d-h	0.05gh
	Arginine + fulvic acid	31.65 h	1.07a-d	0.39def	1.09b-e	1.36a-e	13.46d-g	1.47fgh	2.83c-g	0.06fgh
	Glutamine + humic acid	44.40c-g	0.97b-f	0.45b-e	0.75de	1.20b-g	15.13c-g	1.62e-h	1.62fgh	0.07fgh
	Glutamine + fulvic acid	40.32e-h	1.03a-e	0.38def	1.01cde	1.03c-i	10.99d-g	1.49fgh	3.62bcd	0.10e-h

Similar letter(s) in each column shows significant differences based on the LSD test.

* Peroxidase is expressed in $\mu\text{M H}_2\text{O}_2$ consumed/min/mg protein.

plants, the plants treated with arginine + fulvic acid, and those treated with glutamine + humic acid had the lowest POD activity (Table 3). Eventually, as the comparison of the means revealed (Table 4), the highest POD activity was related to 'Arbequina × glutamine + fulvic acid' and the lowest to 'Zard × arginine + fulvic acid'.

It was revealed by the analysis of variance that the simple effect of organic acids was significant ($P \leq 0.01$) on antioxidant capacity, but the simple effects of cultivar and the interactive effects of 'cultivar × organic acids' were not significant on this trait (Table 1). The comparison of the means showed that the plants treated with arginine had the highest and those treated with glutamine had the lowest antioxidant capacity (Table 3).

4. Discussion

In Jami et al.'s (2014) study, the highest value of total soluble solids (TSS) was 19.21°Bx obtained from 'Arbequina' and the lowest was 16.19°Bx obtained from 'Conservolea'. The Brix value shows the ratio of the soluble solids content in an aqueous solution to total solution weight. In other words, it represents the weight percentage of the solid content of a solution. Therefore, the higher

Brix value of a solution indicates more soluble solids content and less water. (Noktesanj et al., 2018). Ahmadi et al. (2019) reported that the higher content of soluble sugars was related to 'Zard' and the lowest amount to 'Conservolea'. The higher sugar content of 'Zard' is supported by our study, too.

The application of the glutamine amino acid to 'Arbequina' can greatly increase its oil content. The most important factor in the final quality of olive fruits and oil is the cultivar. Olive oil characteristics and content, as well as the efficiency of oil extraction, are all influenced by the cultivar (Fedeli, 1977). Based on Hamidoghli et al.'s (2008) oil content is cultivar-dependent so that oil percentage significantly differed in the three olive cultivars of 'Roghani', 'Zard', and 'Lechino'. Najafian et al. (2008) reported that the treatment of cultivar had a significant impact on oil content and 'Koroneiki' and 'Mission' had the highest and the lowest oil contents of 71.3% and 63.8%, respectively. Jami et al. (2016) found that 'Amygdalolia' had the highest and 'Koroneiki' had the lowest oil content.

Lazovic et al. (1999) found that there are significant differences in protein content among olive cultivars and parts of plant. They reported that the protein content of olives buds ranged from 4.72 to 6.74% and the fruit protein content was 1.5–2.61%. The highest protein content was in olive buds, then in leaves, and the lowest

protein content obtained in fruits. Our results corroborate this report. A field study supported that the application of compounds containing free amino acids can increase the protein content of pea grains by increasing their nitrogen percentage (Mahmoudi, 2012).

Researchers differ in their interpretation of the effect of amino acids on increasing protein content. Some authors believe that amino acids in reduced nitrogen forms are preferred by plants for uptake and this form of nitrogen consumes less energy for protein synthesis (Güneş et al., 1996). Others ascribe the main role of the effect of amino acids in nitrogen assimilation and protein synthesis to its impact on enzymes influencing nitrate metabolism in which the application of amino acids causes to increase of nitrate reductase and glutamine synthetase (Liu et al., 2014; Mobini et al., 2014). Foliar application of humic acid caused to improvement of nutrient efficiency and an increase in leaf Zn and Fe contents, which raised carbohydrate and protein production (Sanjari et al., 2015).

Rauthan and Schnitzer (1981) found that humic acid increased Fe, Zn, Cu, and Mn uptake by cucumbers grown in the Hoagland solution. The increased uptake of Fe and Mn can be an appropriate reason for increasing leaf chlorophyll concentration. Xudan (1986) reported that the foliar application of humic and fulvic acids to farm and greenhouse wheat plants increased their leaf chlorophyll content. The fact that the application of amino acid increased chlorophyll content means that the more appropriate nutritional and environmental conditions, including nutrients, sunlight, moisture, pests, and diseases, are for the plant growth, the more capable the plant will be in synthesizing leaf chlorophyll and producing energy. So, factors that improve these conditions will probably influence chlorophyll content. It should be mentioned that leaf chlorophyll content depends on the plant's genetic and inherent properties so that the leaf chlorophyll content of different cultivars varies with their genetic characteristics (Franco-Mora et al., 2005). It has been established that humic acid increases photosynthesis activity by increasing the activity of the Rubisco enzyme (Delfine et al., 2005). The application of arginine to wheat increased its chlorophyll *a* and *b* (Sairam et al., 2002). Our results support these reports so that the application of glutamine and arginine alone or along with humic acid increased olives leaf chlorophyll.

Carotenoids are the precursor of vitamin A and retinoids and play a significant role in humans and animals' life. These compounds also act as antioxidants, enhance immunity, inhibit mutation, and perform as the precursors of pigments in mammals (Bakó et al., 2002). According to Mania-Djebali et al. (2012), olive cultivars had different chlorophyll and carotenoid contents, and the highest concentration of pigments (6.22 mg/kg chlorophyll *a* and 3.82 mg/kg carotenoid) was recorded in cv. 'Sredki' and the lowest in cv. 'Chladmi'. These pigments in olive oil act as a pro-oxidant in the presence of light and an antioxidant in darkness (Psoimiadou and Tsimidou, 2002). In a study on phenol content and carotenoid compounds of rose fruits, Marie et al. (2005) reported that the contents of carotenoids depended on species and cultivar, supporting the results of the present study.

Anthocyanins are mainly responsible for the dark color of very ripen fruits. This stage of maturity is signified by a significant decrease in chlorophyll content (Vlahov, 1992). Gómez-Rico et al. (2008) reported that in all studied cultivars of olive, cyanidin-1,3-rutinoside was the most abundant anthocyanin, which was about 1050 mg/kg in the fruits of 'Morisca' and 3240 mg/kg in 'Cornicabra' at the black maturity stage. The Begonia semperflorens L. plants exposed to stress exhibited a higher level of anthocyanin content, which is related to the photoprotection role of anthocyanin in directly removing ROS during oxidative stress (Zhang et al., 2010). Gendy et al. (2012) and Ahmad et al. (2011) reported that humic acid increased anthocyanin content in the petals of rose. This might have been related to the effect of humic acid

(a derivative of phenolic compounds) as a precursor of anthocyanin synthesis (flavonoid structure). It is believed that the anthocyanin content of olives increases with fruit maturity, but its content decreases with fruit fermentation, although this trait is highly cultivar-dependent (Aprile et al., 2019).

Phenolic compounds influence the aroma and taste of olive oil and are an index to assess their stability (Soufi Emami et al., 2013). The concentration of phenolic compounds in olive oil is strongly influenced by cultivar (Vinha et al., 2005). Alizadeh Ahmadabadi et al. (2017) showed that the application of humic acid increased total polyphenols in *Echinacea*. Humic acid increased total polyphenols of marigold, too (Abedini et al., 2015). An increase in the phenolic compounds is directly related to the increase in plant carbohydrates. Since carbohydrates are the building block required for the synthesis of phenol compounds, an increase in their quantity means an increase in the substrate for phenolic compounds (Nguyen et al., 2010). Similarly, we observed that sugar content was increased in 'Zard'. The highest polyphenol content among both simple and interactive effects was related to 'Zard', which is consistent with the reasoning mentioned above. Likewise, it has been reported that plants cannot simultaneously allocate resources to both growth and defense and there is a competition between proteins and polyphenols in plants for the precursors typically involved in their biosynthesis. On the other hand, organic acids (e.g., humic acid) act as precursors or activators of plants and secondary compounds in plants, thereby increasing total polyphenols content (Viti et al., 1989). Aprile et al. (2019) found that like anthocyanins, polyphenols were also in a greater quantity in olive ripen fruits than in immature fruits so that their content in mature fruits was over twice more than immature fruits.

A source of H₂O₂ synthesis in plant cells is the SOD enzyme, which converts superoxide radicals to H₂O₂ by dismutation (Arora et al., 2002). SOD is regarded as a key enzyme in plants' antioxidant defense system because it controls the concentration of superoxide anions and H₂O₂ in plants (Mozaffari and Asadollahi Kesarizi, 2011). Since CAT plays an effective role in plant resistance to stress by scavenging H₂O₂, its higher activity can be translated to more resistance of the plant. As a protective enzyme in the plant's antioxidant defense system, CAT is involved in not only the decomposition of H₂O₂ (Laurer, 2003) but also retarding senescence and preventing cell wall degradation (Jiang and Zhang, 2001).

Researchers argue that POD is associated with the physiological damages of heat stress to plants (Chaitanya et al., 2001; Gulen and Eris, 2004) so that its activity is increased at high temperatures. Certain POD isomers consume phenolic compounds and H₂O₂ during the biosynthesis of secondary compounds required for plant growth and development (Gaspar et al., 1982; Chaitanya et al., 2001). Mittova et al. (2004) reported that POD neutralizes the toxic effect of H₂O₂.

Various studies have reported the antioxidant role of polyamines, nitric oxide, and proline, which are the products of the arginine amino acid (Liu et al., 2000; Nasibi and Kalantari, 2009). These compounds are involved in reducing ROS and lipid peroxidation. Glutamine, arginine, vitamins A, C, and E, and polyphenols are some examples of antioxidants. Such antioxidant enzymes as SOD, CAT, glutathione peroxidase, and glutathione reductase are also involved in scavenging free radicals (Neri et al., 2010). The activity of antioxidant enzymes is related to resistance to environmental stresses in many plant species, including olive (Ennajeh et al., 2009). It has been reported that the maturity stage has a significant impact on increasing antioxidant content (Denis et al., 2010). Aprile et al. (2019) reported that as olive fruits were maturing, both their phenolic compounds and antioxidant activity were increased.

5. Conclusions

Based on the results, 'Zard' had the highest Brix value, fruit protein content, carotenoid, anthocyanin, and phenolics. But, the highest oil content, CAT and POD activity were obtained from 'Arbequina'. The highest fruit protein content, chlorophyll *a*, and total chlorophyll were observed in plants treated with arginine + humic acid and the highest anthocyanin and phenolics content in the plants treated with humic acid. The treatment of arginine was related to the highest antioxidant capacity and SOD activity. The highest activity of CAT and POD was obtained from the treatment of glutamine + fulvic acid and the highest oil content from the interaction of 'Arbequina × glutamine'. Overall, the results showed that the foliar application of organic acids to olive trees can positively influence the biochemical properties of the olives. Finally, it can be concluded that Arbequina cultivar produced the most oil when foliar sprayed with glutamine.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Competing Interest

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