

The Beneficial Effect of Spraying Some Antioxidant Vitamins on Fruit Quality, Oil Composition and Improving Oil Characteristics of Picaul Olive

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Abstract: A month before harvest, Picaul olive trees were sprayed with three antioxidant vitamins namely, α -tocopherol, nicotinamide and ascorbic acid individually each at 30, 60 and 90 mg/l. The effect of the antioxidants on fruit physical and chemical properties, also oil characteristics and fatty acid composition were studied. Generally, the antioxidant vitamins, especially α -tocopherol at 60 mg/l and nicotinamide at 90 mg/l increased fruit weight and enhanced fruit physical properties such as fruit length and pulp weight. While ascorbic acid at 90 mg/l followed by α -tocopherol at 30 mg/l and nicotinamide at 90 mg/l improved fruit chemical properties (soluble solid concentration, fruit oil content, total phenol in the fruit and radical scavenging activity), also these treatments reduced acidity percentage compared with the untreated trees. Moreover, the same treatments showed a positive effect on oil characteristics, since they increased total phenol content in the oil, radical scavenging activity, also decreased the acid value of the oil, peroxide value and saponification value. The three antioxidant especially ascorbic acid followed by α -tocopherol improved the total fatty acid composition as unsaturated fatty acid (palmitic acid, palmitoleic acid, oleic acid, linoleic acid and linolenic acid) in comparing with the control.

Key words: Antioxidants • α -tocopherol • Nicotinamide • Ascorbic acid • Picual olive • Fruit properties • Oil properties • Fatty acid composition

INTRODUCTION

The olive tree (*Olea europaea* L.) is native to the Mediterranean basin and parts of Asia Minor. The olive fruit can be consumed either as the fully ripe black fruit or as the unripe green fruit that used for pickling or oil extraction. The olive oil sector plays an important role in many countries economy, providing both employment and export revenue especially in the Mediterranean area. The fruit and compression extracted oil have a wide range of therapeutic and culinary applications. Olive oil is a natural fruit product of fine aroma, pleasant taste and has high nutritional values and is considered as most useful edible oil in the world due to its nutrient contents and well tolerated by the stomach. It provides beneficial effects against ulcer, gastritis and colon cancer [1]. The chief active components of olive oil include oleic acid, phenolic constituents and squalene. The main phenolics include hydroxytyrosol, tyrosol and oleuropein, which occur in highest levels in virgin olive oil and have demonstrated

antioxidant activity. Antioxidants are believed to be responsible for a number of olive oil's biological activities [2]. Antioxidants is designing chemicals, when added in small quantities to a materials, react rapidly with the free radical intermediates of an auto-oxidation chain and stop it from progressing. Recently, there has been increasing interest in oxygen-containing free radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders [3]. Accordingly attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them and on the mechanisms of their action [4]. It has also been reported that plants with high levels of antioxidants, whether constitutive or induced have a greater resistance to such oxidative damage [5]. The primary components of this antioxidant system include carotenoids, ascorbate, glutathione, vitamin E (α -tocopherols) flavonoids, phenolic acids, other phenols, alkaloids, polyamines, chlorophyll derivatives, amino acids and amines and miscellaneous

compounds. Ascorbic acid (vitamin C) is a small water-soluble antioxidant molecule which acts as primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide and considered the most important reducing substrate for H₂O₂ removal [6-9]. Ascorbic acid has been suggested as a bioregulator of plant growth and development [10].

Nicotinamide (NIC), also called vitamin B3 or vitamin pp, functions as a plant growth factor to increase the dry weight of plants and fresh fruit through improving photosynthesis, stimulating CO₂ absorption and restraining respiration [11, 12]. Furthermore, recent research reported that NIC in plants might be related to their defense mechanisms. α -Tocopherols (vitamin E) are lipophilic antioxidants synthesized by all plants. α -Tocopherol found in green parts of plants scavenges lipid peroxy radicals through the concerted action of other antioxidants [13, 14]. Further, tocopherols were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O₂ in chloroplasts [15, 16]. A positive effect of antioxidant treatments was found on vegetative growth, productivity and oil content of many crops, lemongrass [17], sunflower [18], Faba bean [19] and *Zea mays* plants [20]. So, the aim of this study is to investigate the effect of three antioxidants namely α -tocopherol, nicotinamide and ascorbic acid on fruit Physical and chemical characteristics, oil content, phenols content, oil composition and radical scavenging activity of Picual olive.

MATERIALS AND METHODS

The present study was carried out during two successive seasons 2007 and 2008 on Picual olive trees, at a private orchard located at El-Saff district, Giza governorate, Egypt. The olive trees were sprayed with solution of some antioxidant vitamins namely α -tocopheryl acetate, nicotinamide and ascorbic acid a month before harvest date at 30, 60 and 90 mg/l concentrations for each one, while control trees were sprayed with water only. The tested trees were 20 years old, similar in growth vigor and subjected to the common horticultural treatments. Three replicate trees for each treatment were used and the complete randomized design was arranged. About 50 fruits were picked randomly from all sides of each tree at 18th of October to determine their properties. The results were means of the two seasons.

Physical Properties Assessments: Fruit weight (gm), pulp weight (gm), length (cm), diameter (cm) and moisture percentage in the fruit were measured. Fruit samples were ground in an electric blender for freshly prepared juice, soluble solid concentration was measured using hand refractometer [21].

Chemical Properties Assessments: Total acidity was determined by titrating using 5ml of the juice against 0.1 sodium hydroxide using phenolphthalein as indicator [21]. The oil percentage in the dried flesh samples was determined according to AOAC [22]. The extracted oil was analyzed for acid value (%), peroxide value meq./kg and saponification value according to A.O.A.C. [23].

Determination of Fatty Acid by GLC: Methyl esters of fatty acid of olive oil were prepared by methanol-sulphuric acid method [24].

Gas chromatographic analysis of the methyl esters was Flam Ionization Detector (FID) under the following condition capillary column (HP-INNO wax polyethylene glycol) the column temperature was programmed from 70 to 220°C at the rate of 4°C/min the column detector temperature was 280°C. The flow carrier gas, 2 ml/min N₂, 30ml/min, H₂ and air 300 ml/min. Total phenol content: A procedure of folin-ciocalteu was adopted for determination of phenols in methanolic extract of olive fruits flesh and oil [22].

Radical Scavenging Activity in Olive Fruits Extract and Oil Samples by DPPH Test:

Radical scavenging activity of olive fruits extracts was measured by slightly modified method of Brand-Williams *et al.* [25]. Radical scavenging activity in olive oil samples were determined by DPPH test according to reported procedure by Kalantzakis *et al.* [26]. One milliliter of oil solution (10% w/v) was added to 4 ml of freshly prepared DPPH [1, 1-diphenol-2-picrylhydrazyl] solution (0.1 mm) in a 20 ml test tube, which was immediately closed and vigorously for 10s in a vortex apparatus. Ethyl acetate was used as a solvent. Absorbance of the mixture was measured after 30 min at 515 nm and the percent of inhibition was calculated by the following formula:

$$\text{Inhibition \%} = [(A_B - A_A) / A_B] \times 100$$

Where:

A_B = absorption of blank sample (t= 0 min).

A_A = absorption of tested extract solution (t= 30 min).

Statistical Analysis: The design for this work was a Completely Randomized Design (CRD) with three replicates. Data were analyzed with the analysis of variance (ANOVA) procedure of M-STATIC program [27].

RESULTS

Fruit Physical Properties: As for fruit weight, (Fig.1A) it is clear that all α -tocopherol concentrations also the highest and moderate concentrations of nicotinamide treatments increased olive fruit weight. This increment was significant with the highest concentration of α -tocopherol (90 mg/L) which recorded the heaviest fruit (7.13 g.) followed by nicotinamide at 60 mg/L comparing with the untreated trees (control) which recorded 6.05 gm for fruit weight. In this respect, it is observed that the fruit weight was increased with the two higher concentrations (60 and 90mg/l) of α -tocopherol and nicotinamide than the low one and no significant differences were detected between the two higher concentrations (60 and 90 mg/l) of either α -tocopherol or nicotinamide. On the other hand, ascorbic acid did not show any positive effect on fruit weight, but gave similar statistical value of the control.

Regarding fruit length, (Fig.1B) more or less similar results of fruit weight were obtained for fruit length, since nicotinamide and α -tocopherol treatments improved fruit length comparing with the control. However, nicotinamide at 60 mg/l gave the highest value of fruit length followed by α -tocopherol at 90 mg/l. Also, it is noticed that fruit length was increased with 60 and 90 mg/l concentrations of both α -tocopherol and nicotinamide than 30 mg/l of them. Ascorbic acid treatments did not significantly differ than the control, although the fruit length tended to increase by decreasing ascorbic acid concentration.

Concerning fruit diameter, (Fig.2A) antioxidant treatments had insignificant effect on this parameter, although the diameter values tended to increase by different treatments than the control.

Pulp weight was improved by α -tocopherol and nicotinamide treatments, (Fig.2B). In this respect, the highest value of pulp weight was obtained by α -tocopherol treatment at 90 mg/l. However, pulp weight was significantly increased than the control with the all concentrations of α -tocopherol and nicotinamide at 60mg/l. While ascorbic acid treatments gave the similar with the control at the statistical stand point.

Concerning moisture content in the olive fruits, (Fig.3) there was no significant effect for different antioxidants treatments on moisture percentage. However,

α -tocopherol and nicotinamide treatments slightly increased fruit moisture content in compared with the control.

Fruit Chemical Properties: Regarding soluble solids concentration (SSC), most of antioxidants increased this parameter (Fig.4A). In this concern, no constant trend was detected among different concentrations of α -tocopherol and nicotinamide, while S.S.C. was increased by increasing ascorbic acid concentration. However, α -tocopherol at low concentration (30 mg/l) and ascorbic acid at high concentration (90 mg/l) recorded the higher values of soluble solids concentration.

Acidity percentage was significantly reduced due to spraying antioxidant treatments comparing with the control, (Fig.4 B). In this concern, no constant trend was observed among different concentrations of the three antioxidants. However, the low and high concentrations (30 and 90mg/l) of the three antioxidants gave lower values than the medium one.

As for total phenols content in olive fruit (Fig.5A) it is observed that this parameter was significantly increased with 30mg/l of α -tocopherol and the high concentration (90 mg/l) of both ascorbic acid and nicotinamide which increased total phenols than the control by about 34.69, 29.08 and 27.55% for α -tocopherol, ascorbic acid and nicotinamide, respectively. In respect to radical scavenging activity in the fruit, (Fig.5B) all concentrations of the three antioxidants improved and increased radical scavenging activity of the fruits than the control. However, the high concentration (90mg/l) of ascorbic acid and nicotinamide, also 30mg/l of α -tocopherol recorded the higher values of radical scavenging activity and increased it than the untreated trees.

Oil Characteristics: Regarding fruit oil content (Fig.6) all concentrations of the three antioxidant vitamins increased oil content in the fruit especially the highest concentration of nicotinamide (90mg/l) which gave the maximum value of this parameter, followed in decreasing order by α -tocopherol at 30mg/l and ascorbic acid at 90mg/l.

All antioxidants increased the total value of the unsaturated fatty acids especially ascorbic acid which recorded the highest value followed by α -tocopherol, while the lowest value was recorded by the untreated trees. However, ascorbic acid treatments gave the maximum value of oleic acid and palmitoleic acid,

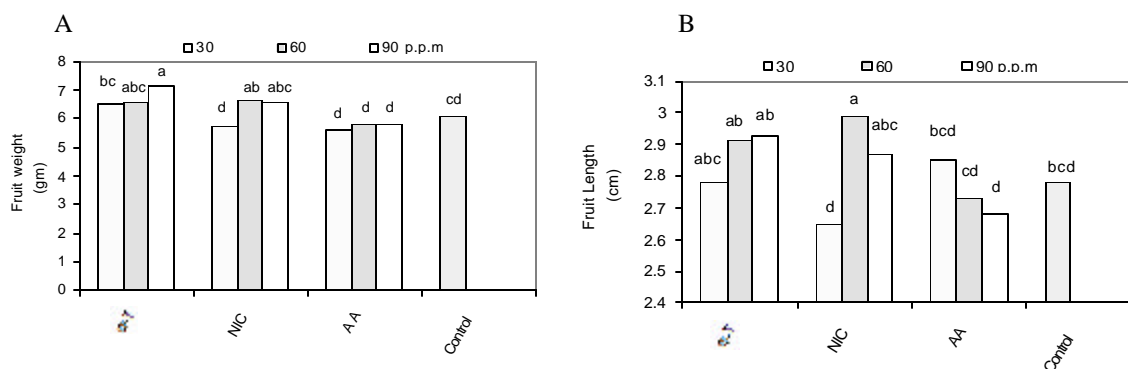


Fig. 1: Effect of different concentrations of antioxidant vitamins applied as spray on fruit weight and length

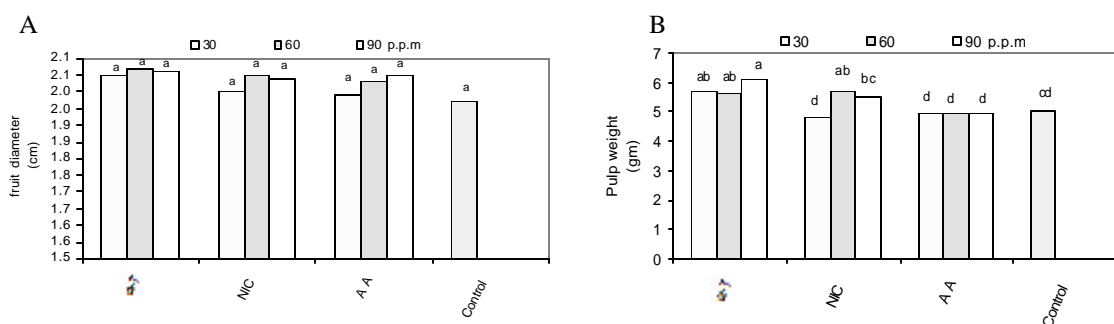


Fig.2: Effect of different concentrations of antioxidant vitamins applied as spray on fruit diameter and pulp weight

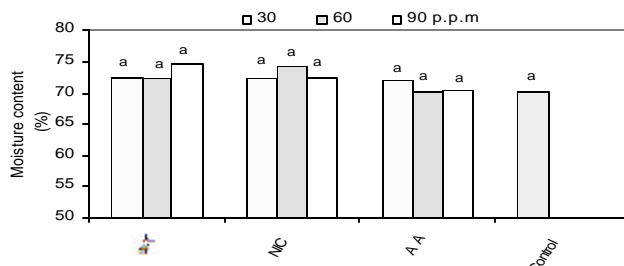


Fig. 3: Effect of different concentrations of antioxidant vitamins applied as spray on fruit moisture content Fruit chemical properties

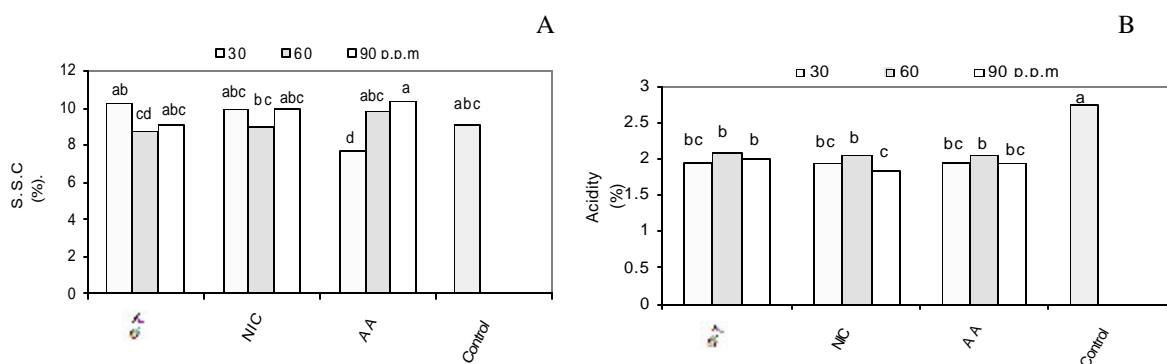


Fig. 4: Effect of different concentrations of antioxidant vitamins applied as spray on fruit soluble solid concentration and total acidity

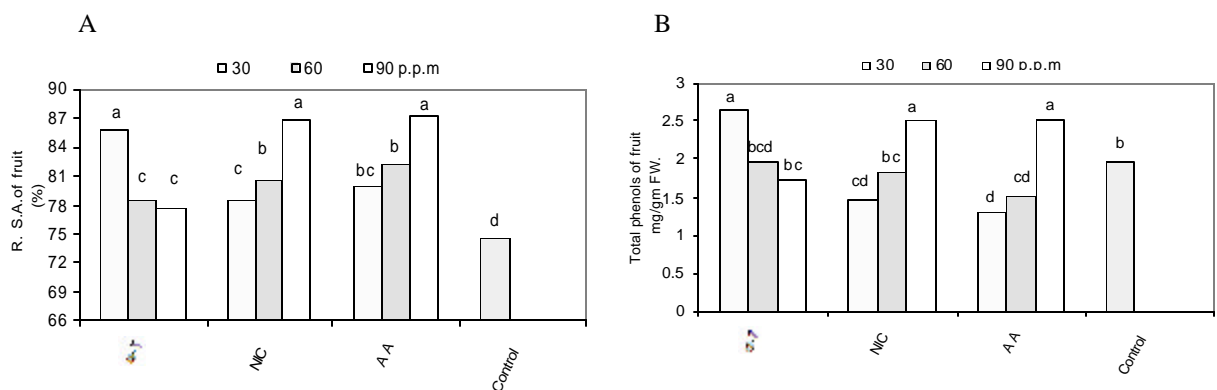


Fig. 5: Effect of different concentrations of antioxidant vitamins applied as spray on fruit total phenols content and radical scavenging activity

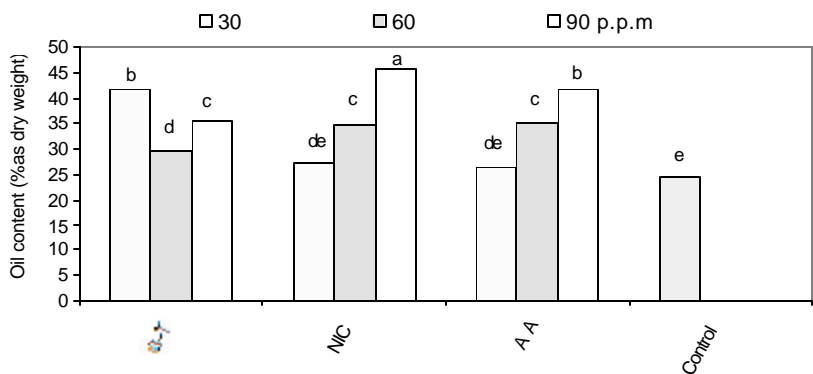


Fig.6: Effect of different concentrations of antioxidant vitamins applied as spray on fruit oil content

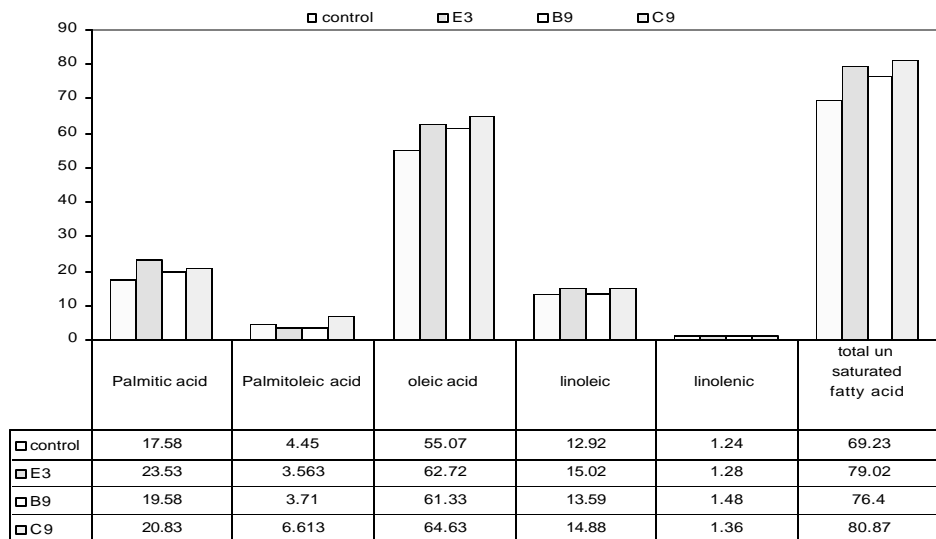


Fig. 7: Effect of different concentrations of antioxidant vitamins applied as spray on Fatty acids composition

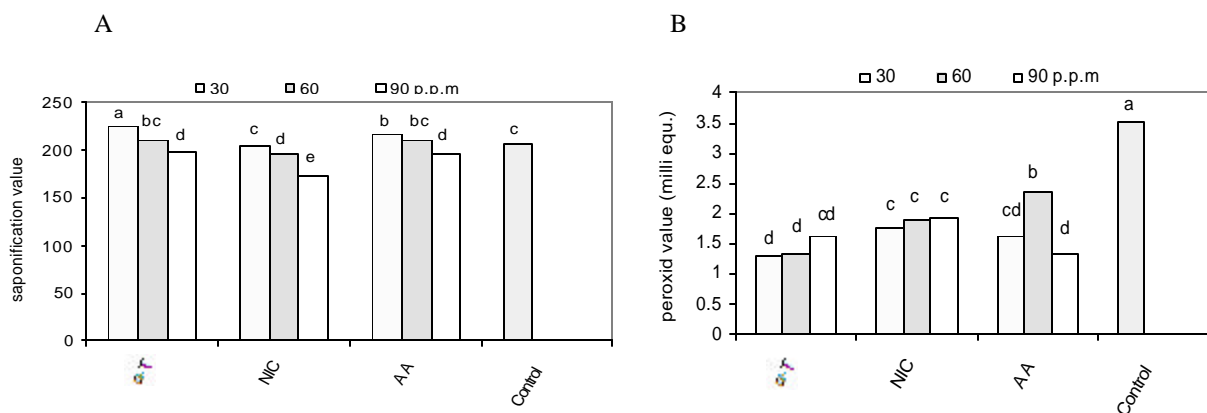


Fig. 8: Effect of different concentrations of antioxidant vitamins applied as spray on fruit oil acid and peroxide value

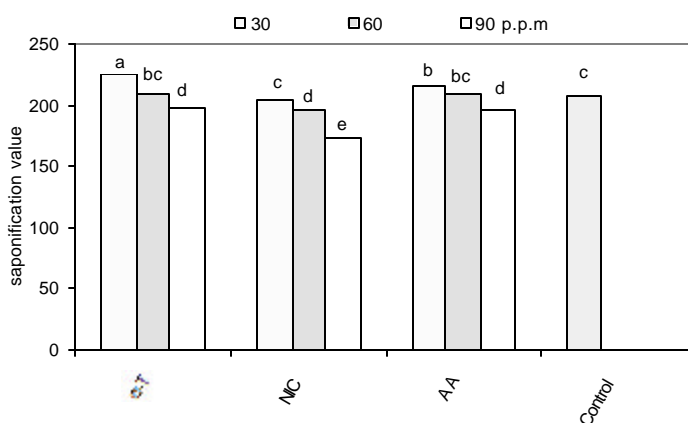


Fig. 9: Effect of different concentrations of antioxidant vitamins applied as spray on fruit oil saponification value

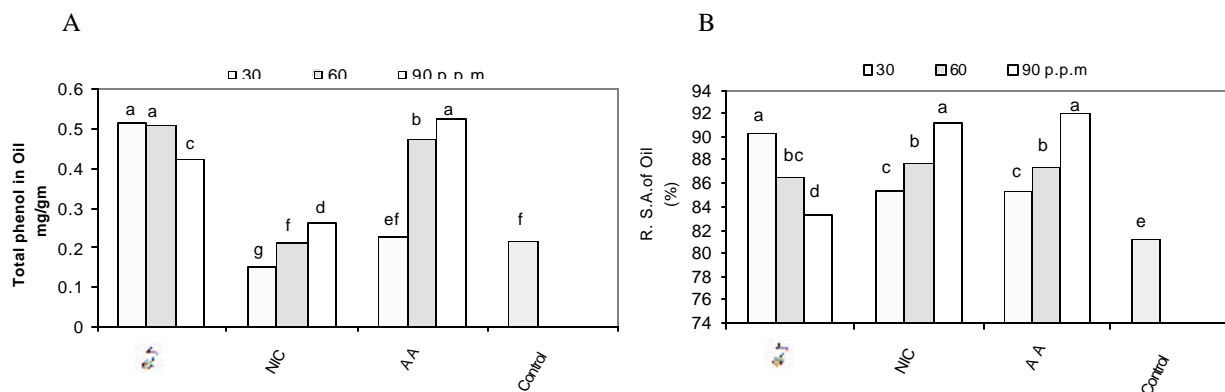


Fig.10: Effect of different concentrations of antioxidant vitamins applied as spray on fruit oil phenols content and radical scavenging activity

while α -tocopherol followed by ascorbic acid recorded the higher values of palmitic acid and linoleic acid. On the other hand, the maximum value of linolenic acid was obtained by nicotinamide treatments (Fig. 7).

The oil acid value (Fig. 8A) was significantly reduced with all antioxidant treatments in compared with the control. However, nicotinamide at 90mg/l gave the lowest value followed in increasing order by ascorbic acid at all concentrations.

Concerning peroxide value of the oil (Fig. 8B) it is clear that all antioxidants reduced this value compared with the control especially when trees treated with α -tocopherol at 30 and 60 mg/l and ascorbic acid at the high one, which gave the lower values.

Regarding saponification value of the oil (Fig. 9) it is observed that 90 mg/l of the three antioxidant vitamins gave the lower value in compared with the two other concentrations. However, nicotinamide at 90mg/l gave the lowest significant value in compared with all the other treatments including the control followed in decreasing order by the same antioxidant at 60mg/l and 90mg/l of both ascorbic acid and α -tocopherol.

Total phenols content in the oil (Fig. 10A) were increased by antioxidant treatments especially ascorbic acid at 90mg/l and α -tocopherol at the low and medium concentrations which recorded the high significant values comparing with the other treatments included the control.

As for oil radical scavenging activity (Fig. 10B) all antioxidant treatments increased this determination and there was a gradual increment corresponded with increasing the concentration of the spraying solution of ascorbic acid and nicotinamide. However, the high concentration (90mg/l) of ascorbic acid, nicotinamide and the low one of α -tocopherol (30mg/l) increased radical scavenging activity than the control by about 13.3, 12.3 and 11.3% for ascorbic acid, nicotinamide and α -tocopherol, respectively.

DISCUSSION

The positive effect of antioxidant vitamins on olive fruit weight and fruit physical properties are agreed with the results obtained by Radi *et al.* [28] who found that nicotinamide treatments increased growth and yield of *Vicia faba* L. plants. Similar results were obtained by Gamal El-Din [18] who sprayed sunflower plants with different concentrations of antioxidant vitamins (thiamine, nicotinamide and ascorbic acid) and found that all

treatments led to significant increase in seed yield per plant and seed weight. Also El-Bassiouny *et al.* [19] reported that spraying faba bean plants with α -tocopherol, ascorbic acid or nicotinamide increased number of seeds/plant, seed yield (g /plant) and seed weight. They added that the maximum yield was obtained due to α -tocopherol at 200 mg/l and nicotinamide at 50 mg/l. In this concern, our obtained results may be due to the effect of antioxidant treatments on increasing total carbohydrate in different plant organs as explained by Mohamed *et al.* [29] on wheat plant, Gharib [30] on *Tagetes minuta* L. plants and El-Bassiouny *et al.* [19] on Faba bean plants. However, Gharib [30], Sahu *et al.* [31] and Hammam *et al.* [32] reported that antioxidant treatments enhanced protein synthesis and delayed senescence. On the other hand, Hathout *et al.* [12], Hassanein *et al.* [20] and Deyab [33] explained that antioxidant vitamins had a stimulating effect on growth development and some metabolic activities of plants, probably due to the increasing of endogenous hormones (auxins and gibberellins).

As for the effect of antioxidant on oil percent in olive fruit, the obtained results are in agree with those of Reda and Gamal El-Din [34] who found that 50 mg/l of ascorbic acid spraying on chamomile plants gave the maximum pronounced increases in the percentage of essential oil. On the other hand, Gamal El-Din [18] reported that the percentage of oil was increased with all treatments of antioxidant vitamins (nicotinamide, ascorbic acid and thiamine) on sunflower plants. Also Tarraf *et al.* [17] reported that nicotinamide and ascorbic acid treatments caused a promotive effect on increasing the essential oil percentage of lemongrass plants. They added that single application of ascorbic acid realized the higher content of the essential oil yield than nicotinamide treatment. In this respect, ascorbic acid was known as an essential oil biosynthesis involved in the formation of isopentenyl pyrophosphate which, on further condensation steps, forms cyclic terpenoids [35]. Therefore, it could be pointed out that exogenous application of ascorbic acid had a regulating effect on essential oil production of olive.

The results of antioxidant treatments on increasing antioxidation components in fruit and oil of olive are agreed with those obtained by Reda and Gamal El-Din [34] and Ayad [36] who reported that total phenol was significantly increased by antioxidant vitamin treatments such as ascorbic acid, nicotinamide and thiamin. They added that antioxidant vitamins enhanced metabolic processes in the direction of increased total phenols and

total indols in chamomile plants. In this concern, the use of antioxidant could be postulated as a key factor to control the oxidation at the membrane levels, limiting increase in hydroperoxide and lipid radical content [37, 38], this may explain the role of antioxidant vitamins in reduce oxidation process through the plant tissues. El-Bassiouny *et al.* [19] found that antioxidant treatments reduced lipid preoxidation and oxidative enzymes such as polyphenol oxidase, peroxidase, catalase and superoxide dismutase activities. However, Gupta and Data [10] and Asada [37] found that α -tocopherol application suppressed membrane lipid peroxidation and plasma membrane permeability. Also, Hussein *et al.* [39] reported that α -tocopherol deactivates photosynthesis-derived reactive oxygen species (mainly O_2 and OH) and prevents the propagation of lipid peroxidation by scavenging lipid peroxyradicals in thylakoid membranes. On the other side, ascorbic acid was known to be involved in oxidation reduction system as electron donor and acceptor in the photosynthetic process [40]. Also, ascorbic acid was reported to scavenge the free radicals which caused increase in the oxidation in plant tissues [41]. In this concern, Shalata and Neumann [6] reported that ascorbic acid treatment in different plant species reduced lipid peroxidation by directly scavenging active oxygen species. On the other hand, nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis as well as activation of peroxide and free radical-degrading enzymes [38] However, Robinson [40] stated that (nicotinamide is a constituent of two co-enzymes termed NAD and NADP involved in many metabolic processes).

In our study, antioxidants had a positive effect on fatty acid compositions as unsaturated fatty acids, these results are in the same line with those obtained by Gamal El-Din [18] who reported that nicotinamide, ascorbic acid or thiamine treatments increased unsaturated fatty acids especially lenoleic acid of sunflower oil as compared with those of saturated ones, also added, that ascorbic acid treatment caused the highest value of total unsaturated fatty acids (oleic and linoleic acid). In this respect, Shahidi [42] stated that (increasing oleic acid percentage improved oil quality of the plants).

CONCLUSION

From the abovementioned results, it could be concluded that antioxidant vitamins had a positive effect on fruit physical and chemical characteristics, oil

content and oil composition of Picual olive. However, α -tocopherol and nicotinamide at 60 and 90 mg/l, respectively increased fruit weight and enhanced fruit physical properties. While as, ascorbic acid at 90 mg/l followed by α -tocopherol at 30 mg/l and nicotinamide at 90 mg/l improved fruit chemical properties, fruit oil content and reduced acidity percentage compared with the untreated trees (control). Also these treatments showed a positive effect on oil characteristics such as oil total phenol, radical scavenging activity, also decreased oil acid value, peroxide value and saponification value. The three antioxidants especially ascorbic acid at 90 mg/l followed by α -tocopherol at 30 mg/l improved the total fatty acids composition as unsaturated fatty acids in compared with the control trees.

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