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The Effect of Sulfur-Containing Humic Acid on Yield and Nutrient Uptake in Olive Fruit

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Abstract

The combined application of organic and mineral fertilizers can be a proper way of nutrition management to increase the yield and quality and to mitigate environmental impacts of chemicals and the pertaining costs. The impact of different rates of sulfur-containing humic acid was studied on yield and nutrient uptake of the fruits of olive "Zard" three-year-old trees in a trial based on a Randomized Complete Block Design with three replications in Shariati College of Tehran, Iran. The studied treatment was sulfur-containing humic acid at four rates of 0, 20, 25 and 30 $kg \cdot ha^{-1}$, which was applied at two stages. The highest fresh weight, dry weight, chlorophyll *b*, carotenoid, N and Cu contents were related to humic acid rate of 20 $kg \cdot ha^{-1}$. The highest fruit length, chlorophyll *a* and total chlorophyll were observed in control. The highest K, Fe and Mn contents were obtained from plants treated with 30 $kg \cdot ha^{-1}$ humic acid. Plants treated with 25 $kg \cdot ha^{-1}$ exhibited the highest P and Zn contents. All in all, the application of sulfur-containing humic acid at different rates had favorable impacts on quantitative and qualitative traits of olive fruits.

Keywords

Humic Acid, Olive, Sulfur, Yield

1. Introduction

Olive (*Olea europaea* L.) is a species of hot and semi-hot regions originated from Syria, Southern Turkey and Palestine about 3000 BCE. It was domesticated 6000 years ago [1]. Olive is adapted to Mediterranean semi-arid conditions with the capability of growing in arid regions too, so that its cultivation is steadily growing in these regions [2]. Olive cv. "Zard" is a shrub with medium to tall size, circular crown, and relatively large, round fruits with 8 t·ha^{-1} yield.

Organic farming is a system of agricultural production in its chemical fertilizers, pesticides, hormones and additives no synthetic chemical used to enhance fertility. Soil, control pests, diseases and weeds methods non-chemical such as crop rotation, green manure, biological control (And other non-chemical methods of pest and disease control and Weeds) compost and the like are used [3].

Organic crops contain less toxic chemicals than commercial products [4]. Soil organic nutrition is a global strategy to conserve natural fertility of soils through improving soil microorganisms [5]. Combined application of organic and mineral fertilizers can be a proper way of nutrient management to increase yield and quality and to reduce adverse environmental impacts of chemicals and the pertaining costs. Humic substances, e.g. humic acid and folic acid, cover a wide range of mineral organic compounds including amino acids, peptides, phenols, aldehydes, and nucleic acids in a bond with different cations and play an effective role in the improvement of plant growth and development in the composition of substrates and nutritional solutions [6].

Humus compounds of different organic matters contain two important organic acids—humic acid and folic acid. Humic acid is produced by the decomposition of organic matter, especially those with a plant origin, and can be found in soil, coal and peat resulting in the formation of stable, insoluble complexes with micronutrients. Folic acid forms soluble complexes with micronutrients. Humic acid is a mix of very large molecules with the capability of chelating elements and along with folic acid is a very important component of soil humus and is nontoxic to plants, animals and people. Humic acid is adapted to most chemical fertilizers and can be mixed with them. It is fully soluble in water and can be mixed with other liquid fertilizers, and it can be applied by soil application and with pressurized irrigation systems [7].

Humic acid directly acts as a quasi-hormonal compound [8] and indirectly improves soil physical condition, increases the metabolism of soil microorganisms, enhances root and stem growth [9], and increases the uptake of nutrients through its chelating, restoration and membrane infiltration conservation properties [10] [11]. As an organic acid derived from humus and other natural resources, humic acid has quasi-hormonal impacts [12] [13], induces the uptake of nutrients [14] [15] [16] and increases root and shoot biomass [7]. Humic acid is known to improve the uptake of soil nutrients and plant growth. It augments shoot: root ratio by increasing the generation of thin lateral roots in plants [15].

Ramazani *et al.* (2008) stated the consecutive fruit harvest for a long time on the one hand and insufficient nutrient supply to plants on the other hand as the main reasons for yield loss [17]. The buildup of organic matter in leaves is impossible if minerals are not present in photosynthesis process. Each macro element has a specific role in the metabolism of plants growth and development. The flowering of the plants is affected by nutritional status, and the balance between materials the plants get from the air and soil (C:N ratio) is crucially important for flowering. The availability of ions is deeply influenced by pH because it affects their oxidation and solubility (e.g. in the case of phosphorus, sulfur and aluminum) or their controlling bioprocesses. In short run, the restoration of minerals from organic residues is the main, direct source of soluble minerals for soils [18].

Most plants need more phosphorus than sulfur [19]. Oilseed plants are among the species that have a high demand for sulfur. Sulfur is a component of the structures of methionine and cysteine amino acids, so it is involved in protein structure. In addition, it is involved in the formation of vitamins and glucoside and the activation of enzymes. Furthermore, sulfur is a part of phospholipids and thus, it directly contributes to the formation of fats [20].

Excessive use of chemical fertilizers and the consequent environmental pollution have led the mankind towards the use of the natural and organic fertilizers. Humic acid fertilizers improve the crop and yield by enhancing soil organic content and chelating nutrients. The present study was aimed to investigate the impact of sulfur-containing humic acid on quantitative and qualitative properties and the nutrients content of olive "Zard".

2. Materials and Methods

The study was carried out on three-year-old trees of olive "Zard" in Shariati College of Tehran, Iran on the basis of a Randomized Complete Block Design with three replications. The applied treatments included sulfur-containing humic acid (Khorram Bahar Atis company) at four levels of 0 (control), 20, 25, and 30 $kg \cdot ha^{-1}$ used as fertigation at two stages-in late-May after fruit setting (flower shedding and fruit formation) and in September during fruit filling. The recorded traits included yield, fruit fresh and dry weight, diameter and height, and their micronutrient and macronutrient contents.

To determine the yield of a tree, all its fruits were picked and were weighed to be expressed in grams. To measure fruit length and diameter, 10 fruits were randomly selected on each tree and their length and diameter were measured by a digital caliper.

Fresh weight was estimated by weighing the samples with a digital scale. Then, they were oven-dried at 80˚C for 48 hours to get their dry weight.

Chlorophyll *a*, *b* and *a* + *b* and carotenoid contents of the fruits were estimated by Burns *et al.* (1992)'s procedure. Accordingly, 0.5 g of fruit sample was weighed and ground. Then, it was poured into a test tube and was added with 10 ml dimetyl sulfoxide. Next, the samples were over-dried at 75˚C - 80˚C for three hours. Afterwards, 1 ml of the solution was poured into another test tube and was reached to 5 ml by DMSO addition. Finally, their absorptions were read at 480, 663, 645 and 510 nm by a spectrophotometer.

To estimate nutrient absorption rates, the concentrations of N, P, K and micronutrients (Fe, Mn, Zn and Cu) were measured by Kjeldahl, calorimetry, flame photometry and atomic absorption method, respectively.

3. Results and Discussion

3.1. Yield

Results analysis of variance showed that sulfur-containing humic acid influenced yield significantly at the 1% probability level (Table 1). Also, according to means comparison, the highest yield was related to humic acid rate of 25 kg·ha^{-1} and the lowest one to control (Table 2).

The application of humic acid improves soil physical structure, helps soil moisture retention, increases root permeability to water and nutrients, enhances the generation of nucleic acids and amino acids, improves plant enzymatic activity and metabolism, and consequently, improves yield [21]. The application of organic matter along with sulfur accelerates biooxidation of sulfur. Organic sulfur fertilization helps better plant growth by improving the absorbability of soil macro and micronutrients and enhances the quantitative and qualitative yield [22].

3.2. Fruit Fresh and Dry Weight

Analysis of variance revealed the significant impact of sulfur-containing humic acid on fruit fresh and dry weight at the 1% probability level (Table 1). Means comparison showed that the highest fruit fresh and dry weight was obtained from plants treated with 20 kg·ha^{-1} humic acid and the lowest ones from those treated with 30 kg·ha^{-1} (Table 2).

Humic acid improved the fresh weight of shoot and root of maize and pepper [23] [24]. The application of humic acid enhanced dry weight in strawberry [25], maize and oat [26], and wheat [27]. Results reflect the significant impact of foliar

Table 1. ANOVA the effect of sulfur-containing humic acid on measured characteristics of Olive.

S.O.V	df	Yield	Fresh weight fruit	Fruit dry weight	Length fruit	Fruit diameter	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Treatment	3	23,311,549.19**	0.378**	0.158**	12.802**	0.279ns	1.797*	0.458ns	1.495ns	106.015**
Error	6	143,640.69	0.01	0	0.24	0.3	0.26	0.1	0	0.6
Cv		6.273	3.8	11	2.03	3.3	6.96	7.2	5	2.9

**, * and ns: Respectively significant difference and at 5% and 1% and non-significant.

Table 2. Mean comparison the effect of sulfur-containing humic acid on measured characteristics of Olive.

Treatment	Yield	Fresh weight fruit	Fruit dry weight	Length fruit	Fruit diameter	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
C	2814.3c	2.54a	0.78b	26.09a	15.41a	8.48a	4.20b	12.68a	31b
H20	4598b	2.67a	1.13a	25.95a	15.65a	6.89b	5.14a	12.04ab	33.59a
H25	8383.3a	2.09b	0.8b	22.83b	15.05a	6.99b	4.83ab	11.77ab	24.08c
H30	8350a	1.92b	0.57c	22.12b	15.02a	6.92b	4.78ab	10.98b	20.79d

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

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application of different rates of humic acid on the fresh and dry weight of olive fruits. Researchers have reported the significant changes in shoot and root dry weight in plants treated with humic acid [7] [28] [29]. Given the role of sulfur in chlorophyll and chloroplast synthesis, root growth, stomatal opening, and photosynthesis, it can be concluded that higher fresh and dry weight in plants treated with sulfur-containing humic acid was related to the role of this element.

3.3. Fruit Diameter and Length

Analysis of variance showed the significant impact of sulfur-containing humic acid on fruit length at the 1% probability level, but it did not change fruit diameter significantly (**Table 1**). Means comparison showed that the highest length was related to control and the lowest one to humic acid rate of 30 kg·ha^{-1} (**Table 3**). The highest and lowest fruit diameters were obtained from humic acid rates of 20 and 30 kg·ha^{-1} (**Table 2**).

Foliar or powder application of humic acid resulted in longer and heavier roots in carrots and improved the growth of whole plant [30] that confirms our results.

3.4. Chlorophyll a and b, Total Chlorophyll and Carotenoid

As analysis of variance revealed, sulfur-containing humic acid significantly influenced chlorophyll *a* content at the 5% probability level and carotenoid level at the 1% probability level, but it did not change chlorophyll *b* and total chlorophyll content significantly (**Table 1**).

Sladky and Tichy (1959) observed that humic acid improved chlorophyll content of tomato leaves by 63% in plants grown in humic acid containing solution and by 15% in those grown in folic acid containing solution [31]. Foliar application of humic acid at 200 ml·l^{-1} resulted in higher chlorophyll content of bell peppers [32].

Research shows that humic acid stimulates photosynthesizing activity in plants through increasing the activity of rubisco enzyme [33]. Given the critical role of plant hormones, especially cytokinin, in the fixation and increase of chlorophyll [34] and quasi-hormonal compounds (cytokinin) of humic matter [7] [8], the loss of chlorophyll content in plants treated with humic acid in the present study is inconsistent with other studies that show higher chlorophyll content under humic acid application.

3.5. Macronutrients

Results of analysis of variance indicated the significant impact of sulfur-con-

Table 3. ANOVA the effect of sulfur-containing humic acid on macronutrients and micronutrients of Olive.

S.O.V	df	Nitrogen	Phosphor	Potassium	Iron	Zinc	Copper	Manganese
Treatment	3	0.009**	0.006**	0.037**	51,654.527**	1398.083**	33,673.416**	137.46**
Error	6	1E−05	0	0	0.44	1.1	2.25	0.3
Cv		1.052	4.71	0.3	0.33	1.1	0.81	2.2

**, * and ns: Respectively significant difference and at 5% and 1% and non-significant.

taining humic acid on the measured macronutrient levels at the 1% probability level (**Table 3**). Means comparison revealed that the highest N content was related to humic acid rate of 20 kg·ha^{-1} and the lowest one to 30 kg·ha^{-1} humic acid. The highest P content was observed in plants treated with 25 kg·ha^{-1} humic acid. K content exhibited an increasing rate with humic acid so that the highest humic acid rate, *i.e.* 30 kg·ha^{-1}, was related to the highest K content (**Table 4**).

Humic materials improve the synthesis of ion-transporting proteins and thus, increase uptake rate [35] [36].

This mechanism was confirmed by Nardi *et al.* (2000) in a study on MRNA of ion transporters in maize plants treated with humic acid [37]. In addition, humic acid improves the uptake of nutrients and the yield of plants via forming stable complexes with nutrients, especially micronutrients like Fe and Zn [15].

According to Nardi *et al.* (2002), humic acid is a natural polymer that helps the uptake of nutrients directly (as auxin or cytokinin quasi-hormones) or indirectly [7]. The application of humic acid in tomato hydroponic system influenced the uptake of calcium and potassium and the length of shoot and root in plants, but leaves and fruits differed in their contents of these nutrients. Humic acid improves P solubility in soil [38] and reduces K fixation in soil resulting in their higher uptake by plants [39]. The application of humic acid increased leaf N, P and chlorophyll content in tomato "Camarosa" [40].

Humic acid has been reported to stimulate and improve stomatal opening and leaf K content in potato "Golden Delicious" [41], lettuce [42], cucumber [43] and strawberry [44]. Higher K content can be related to the presence of K_2O in humic acid composition and higher acidity of the solution given the fact that K is absorbed better in alkaline medium [45]. Higher alkalinity at higher rates of humic acid can be a reason for greater K uptake and K content in leaves [46].

3.6. Micronutrients

Analysis of variance showed that sulfur-containing humic acid affected the amount of the measured micronutrients significantly at the 1% probability level (**Table 3**). The highest Fe content was measured at humic acid rate of 30 kg·ha^{-1} and the lowest one was observed in control. The highest Zn content was observed in plants treated with 25 kg·ha^{-1} humic acid and the lowest one in those treated with 30 kg·ha^{-1} humic acid (**Table 4**).

Atiyeh *et al.* (2002) reported that the application of humic acid increased the concentration of micronutrients in tomato plants [9]. Humic acid treatment of

Table 4. Mean comparison the effect of sulfur-containing humic acid on macronutrients and micronutrients of Olive.

Treatment	Nitrogen	Phosphor	Potassium	Iron	Zinc	Copper	Manganese
C	0.370b	0.44b	1.28d	87d	66.66d	78.66d	21.80c
H20	0.380a	0.52a	1.48c	102.33c	108.33b	325.66a	22.70bc
H25	0.30c	0.54a	1.50b	242.33b	115.66a	201.66b	23b
H30	0.26d	0.46b	1.52a	366a	95.66c	137c	36a

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

lime soil improved the uptake of Zn, Mn and Cu by maize plants [47]. Humic materials chelate Fe and Zn, resulting in their higher availability to plants [48]. As well, they improve the uptake of water and nutrients by plants through enhancing the permeability of root cells [21].

Rauthan and Schnitzer (1981) reported that the treatment of cucumbers with humic acid improved the uptake of Fe, Zn, Cu and Mn and that higher uptake of Fe and Mn enhanced chlorophyll content [43]. In a study on cut gerbera flowers, Nikbakht *et al.* (2008) observed that humic compounds (500 and 1000 $mg \cdot l^{-1}$) resulted in higher Ca uptake than control [16]. The application of humic acid increased Cu content in cucumber [49]. Our results are consistent with other relevant research.

4. Conclusions

The ever growing population has forced mankind to over-exploit agricultural lands to meet food requirements. Consecutive use of soil has damaged its physical structure, causing the deficiency of nutrients. This deficiency is observed in plants grown in these lands, too. The application of such compounds as humic acid that improves the physical and chemical properties of soil can help us produce high-quality crops and avoid soil erosion. Sulfur-containing humic acid increased the fresh and dry weight and diameter of olive fruits. The better growth and physiological conditions of plants sprayed with sulfur-containing humic acid were related to their higher macronutrient (N, P and K) and micronutrient (Mn, Zn, Cu and Fe) contents. Consequently, we can produce high-quality crops by applying sulfur-containing humic acid.

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