The feasibility for replacement of urea with nitrogen nano-chelated fertilizer in olive *(Olea europaea* L.) orchards

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Abstract

Nitrogen is an important element in the life of a plant. A wide range of nitrogen-containing compounds is available in the world market, with different formulations and efficiencies. Urea is present in most fertilizer applications; however, nano-nitrogen products are becoming popular although there is only limited information on their field efficiency. Thus, this paper studied the foliar application with two sources of nitrogen (urea and nano-chelated nitrogen fertilizer) on 15-year-old 'Zard' olive trees. Fertilizers were used during the bud-swelling stage, before blooming, pit hardening stage, and shortly after harvest of table olive, at the concentration of 2.21 (U_1) and 2.95 (U_2) g urea, 6 (nano-N_1) and 8 (nano-N_2) g nano-chelated nitrogen, corresponding to 1.02 g (U_1 and nano-U_1) and 1.36 g (U_2 and nano-N_2) pure nitrogen per liter. Results revealed that the nano-N_1 treatment increased the fruit set. However, fruit yield efficiency increased using U_1 in both years. Fruit consuming-quality attributes were affected mainly by U_1 treatment. Mineral elements, chlorophyll and carbohydrate contents of leaves were affected during summer and fall by nitrogen treatments. The maximum oil percentage was achieved by nano-N_2 treatment. However, oil yield increased with increasing fruit load as a result of applying U_1. It seems that urea, due to rapid absorption compared with slow-released nano-nitrogen, provided requiring assimilates in the growing season so that especially in the first year the yield and mineral composition improved. However, in the second year, both types of fertilizers led to improved nutrient status. Further research is recommended for application of nano-chelated nitrogen fertilizer in olive orchards.

Keywords: foliar application; mineral elements; nanotechnology; *Olea europeae* L.

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Introduction

Demands for the employing nature-friendly approaches to mineral nutrition management in agro-ecological systems are on the increase. Several methods and practices have been considered to optimize the use of nutrient sources including adopting a technology for nutrient supply with high efficiency (e.g., organic-based fertilization, foliar application, and nano-fertilization), splitting nutrient rates, and adapting the amount of fertilizers to match the needs of trees. It seems that during the 21st century, the adoption of the orchard management systems for

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applying different organic fertilizer compounds will have an important role in improving food security and crop yield for an increasing population around the world and preserving the ecosystem quality.

Olive is an evergreen tree which grows in the Mediterranean climate with relatively rustic and dry conditions. The oil extracted from its fruits is well recognizing for both health benefits and flavor. Foliar application of fertilizers is unquestionably not a new concept to the olive industry, and there are numerous studies dealing with the effectiveness of this approach in olive (Fernández-Escobar et al., 2011; Saykhul et al., 2014). Foliar fertilization is considered as an intrinsic component of nutrition programs in fruit trees aimed to increase nutritional status through short but important periods of nutrient demand, such as flower bud's differentiation, fruit set, and vegetative growth. El Khawaga (2007) reported a synergistic influence of foliar application of nutrients accompanied by girdling on growth and fruiting of Manzanillo olive trees.

Nitrogen is one of the important mineral nutrients, improving yield production in many horticultural crops globally. It has a crucial impact on crop production and quality and also affects the taking up and distribution of other nutrients in fruit trees (Barker and Pilbeam, 2015). Adequate N availabilities to the olive tree enhances its vegetative and reproductive growth, two stages that are of the greatest significance for the productiveness of established orchards and therefore is able to increase fruit and oil yields (Fernández-Escobar et al., 2009). Nitrogen deficiency influences shoot growth, fruit set, and yield in olive trees negatively (Freeman et al., 1994). Urea may be the most widely used source of nitrogen for foliar applications due to its high solubility and low salt index in comparison with other nitrogen forms. It has been shown that urea stimulates the absorption of other nutrients by increasing the leaf tissue permeability. However, its utilization should be low in foliar sprays to avoid leaf necrosis (Etehadnejad and Aboutalebi, 2014; Fernández et al*.,* 2013). In olive trees, it has been reported that foliar application of urea increased the nitrogen content of the leaves (Connell et al., 2000; Fernández-Escobar et al., 2011). The positive effect of winter urea foliar sprays on fruit quality and yield of 'Picual' olive trees has been reported (Abd El Migeed et al., 2017). Other sources of nitrogen can be obtained from different fertilizer compounds which are available in the world market. Among which, nano products are gaining popularity (Kashyap et al., 2015; Wu et al., 2018). These sources, when put to use at low amounts of foliar spray, are perfect accessorial nitrogen bearer with low foliage necrosis and side-effects. Davarpanah et al*.* (2017) indicated that the fruit yield of pomegranate was improved comparably with applications of nano-nitrogen fertilizer at a rate of 1.8 kg N/ha and utilization of urea at a rate of 16.3 kg N/ha. However, there is limited or no literature on nano-chelated nitrogen compared to regular nitrogen-containing compounds, i.e., urea. Thus, our objective was to compare the influence of routinely-used urea with nano-nitrogen formulation on vegetative and reproductive growth attributes of 'Zard' olive.

Materials and Methods

Experimental site, plant materials, and treatments

An experiment was carried out during two successive growing seasons of 2017-2018 in a commercial olive orchard located in Manjil, Guilan province in the north of Iran (49° 25′ E, 36° 44′ N, altitude 396 m). The soil was of clay-loam texture (24 % sand, 36 % clay and 40 % silt) with a pH of 7.39 in water and an EC of 1.03 dS m^{-1}. Experimental trees included 15-year-old olive trees cv. Zard planted in regular rows and spaced at 6 × 8 m and irrigated by a drip irrigation system with normal growth, uniformed in vigor receiving the same horticultural practices. The two sources of nitrogen including urea (NH_2CONH_2) and nitrogen nano-chelated (nano-N) were applied at four stages (with a volume of 5 L per time), namely the bud-swelling stage, before blooming, pit hardening stage, and shortly after harvest of table olive. Nano-N fertilizer was obtained from Khazra Company, Teheran, Iran (http://en.khazra.ir). Fertilizers were used in a foliar application at the concentration of 2.21 (U_1), 2.95 (U_2) g N L^{-1} (urea), 6 (nano-N_1), and 8 (nano-N_2) g N L^{-1} (nitrogen nano-chelated) corresponding to 1.02 and 1.36 g pure nitrogen per liter of each fertilizer formula, respectively. Spraying with water was considered as control.

Vegetative growth and flowering traits

Twenty-one-year-old shoots around the tree at a height of 1.5 m above ground level were chosen randomly and labeled for measuring the parameters. The mean number of inflorescences per shoot, number of flowers per inflorescence, number of perfect flowers, and number of imperfect flowers were recorded at full bloom. The shoot length and the number of leaves per shoot were recorded at the end of the growth season (Fernandez and Gomez, 1985).

Fruit set, yield and fruit quality

Final fruit set following the eight weeks after full bloom was recorded on the labeled shoots. At the green ripening stage fruits were collected from each replicate and weighted to determine fruit yield. Yield efficiency was calculated by dividing yield into trunk cross-sectional in each tree. Twenty fruit from each replicate were randomly selected for measuring the fruit weight, pit weight, pulp weight, and pulp to pit ratio.

Leaves chlorophylls, carbohydrates content and nutrient elements concentrations

The content of chlorophyll, macro- and micro-nutrients, and soluble carbohydrates of leaves were measured twice per each growing season, i.e., in August (during pit hardening) and in October (shortly after harvest of table olives). Leaf samples were collected from the middle part of current year growth (fifth and sixth nodes counting from the top of the shoot) around the tree at shoulder height and cleaned using ethanol 70%. Total chlorophyll content of the leaves was determined by spectrophotometric method (Arnon, 1949) and the carbohydrate content was estimated using the Anthron reagent method (Irigoyen et al., 1992). For determination of the nutrient elements, leaf samples were grounded to pass a 40-mesh screen. Kjeldhal method for the determination of nitrogen (N), colorimetric method for determining boron and phosphor (B and P), flame emission spectrometry method for quantification of potassium (K), and atomic absorption spectrophotometry method for determining the iron (Fe) were used according to (Walinga et al., 1989).

Oil percentage

Oil percentage was determined with dried flesh fruit basis using Soxhlet oil extraction apparatus with Hexane 60 - 80 °C boiling point and the results were expressed as the percentage of dry matter accordingly.

Statistical Analysis

An experiment was carried out based on a randomized block design and each treatment consisted of three replications. Data were statistically evaluated by analysis of variance (one-way ANOVA) using SAS 9.1 (Statistical Analysis System; SAS Institute Inc., Cary, NC, U.S.A.) software. The data of two years have been analyzed separately. Means were compared using the least significant difference (LSD, $p \leq 0.05$).

Results

Reproductive and vegetative traits

Results showed that treatments U_1, U_2, and control gave similar inflorescence numbers per shoot in the first year, but the effects of the treatment U_1 was better in the second year. Trees with no nitrogen application had the highest number of flowers per inflorescence and number of staminate flowers in both years. However, U_1 applied to olive trees increased the number of perfect flowers in the first and second years. In two successive years, no differences among the treatments were found for inflorescence length (Table 1).

Results presented in Table (1) indicate that the vegetative characteristics were significantly affected by nitrogen treatments. Shoots of trees treated with foliar N applications were significantly longer than shoots on control trees. Moreover, U_1 and nano-N_2 treatments increased both the shoot length and the number

Table 1
The effect of Urea and nano-Nitrogen foliar applications on morphological responses of olive tree

Treatment	Inflorescence No.		Flower No.		Inflorescence length (cm)		Staminate flower No.		Perfect flower No.		Shoot length (cm)		Leaves No. per shoot	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
*Control	14.45^{a}	11.11^{b}	19.17^{a}	17.66^{a}	2.68^{a}	2.75^{a}	16.52^{a}	14.56^{a}	2.65^{b}	3.10^{d}	6.00^{d}	7.11^{c}	11.83^{d}	15.50^{d}
U_1	15.46^{a}	14.16^{a}	13.45^{c}	12.56^{d}	2.58^{a}	2.56^{a}	10.05^{c}	6.32^{d}	3.40^{a}	6.24^{a}	12.00^{a}	11.91^{b}	24.00^{a}	23.33^{bc}
U_2	14.45^{a}	12.20^{b}	16.62^{b}	15.41^{b}	2.54^{a}	2.59^{a}	14.33^{b}	11.41^{b}	2.29^{b}	4.00^{c}	9.75^{b}	11.66^{b}	20.16^{b}	25.33^{b}
nano-N_1	8.46^{b}	6.41^{d}	11.5^{d}	10.25^{e}	2.62^{a}	2.45^{a}	9.16^{c}	5.70^{d}	2.34^{b}	4.55^{b}	7.41^{c}	11.16^{b}	14.83^{c}	22.60^{c}
nano-N_2	9.22^{b}	8.16^{c}	15.73^{b}	13.66^{c}	2.59^{a}	2.58^{a}	13.48^{b}	10.41^{c}	2.25^{b}	3.25^{d}	7.00^{c}	14.08^{a}	14.10^{c}	29.16^{a}

*Control, (U_1 & nano-N_1), and (U_2 & nano-N_2) are 0, 1.02, and 1.36 g L^{-1} pure nitrogen, respectively. Means with the same letter in each column were not significantly different using Least Significant Difference test at $p \leq 0.01$.

Table 2
The effect of Urea and nano-Nitrogen foliar applications on yield and oil percentage of olive tree

Treatment	Fruit set (%)		Yield (kg/tree)		Yield efficiency (kg/cm²)		Olive oil (%)		Oil yield (kg/tree)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
*Control	0.69^{c}	0.99^{d}	24.00^{d}	13.00^{d}	0.11^{c}	0.05^{d}	50.66^{c}	51.71^{c}	12.15^{d}	6.72^{c}
U_1	1.20^{b}	2.44^{b}	35.00^{a}	32.40^{a}	0.15^{a}	0.11^{a}	47.50^{d}	43.60^{e}	16.62^{a}	14.12^{a}
U_2	0.70^{c}	1.60^{c}	28.00^{b}	22.06^{b}	0.13^{b}	0.09^{b}	51.33^{c}	50.75^{d}	14.37^{b}	11.19^{b}
nano-N_1	1.92^{a}	3.85^{a}	25.10^{c}	19.50^{c}	0.11^{c}	0.08^{c}	53.10^{b}	52.70^{b}	13.32^{c}	10.27^{b}
nano-N_2	1.10^{b}	1.77^{c}	26.00^{c}	20.00^{c}	0.11^{c}	0.07^{c}	56.50^{a}	55.14^{a}	14.69^{b}	11.02^{b}

* Control, (U_1 & nano-N_1), and (U_2 & nano-N_2) are 0, 1.02, and 1.36 g L^{-1} pure nitrogen, respectively. Means with the same letter in each column were not significantly different using Least Significant Difference test at $p \leq 0.01$.

of leaves per shoot when compared with the other treatments in the first and second years, respectively while the minimum values for those traits were found in control.

Fruit set, Fruit yield and oil percentage

The nano-N_1 foliar application significantly increased fruit set about 2.78 and 3.88 times more in comparison with control trees in the first and second years, respectively (Table 2).

Fruit yield improved in response to nitrogen foliar application with the best results being obtained with the treatment supplying 2.21 g urea in two successive years whereas the lowest yield was recorded in control (Table 2). Also, yield efficiency in U_1 was significantly higher than that of other treatments.

The data indicated significant differences among treatments with the maximum amount of olive oil (56.50- 54.00 %) at nano-N_2 treatment in two years. Oil yield increased with increasing fruit load as a result of applying U_1 in the first and second years, respectively (Table 2).

Fruit physical properties

A statistically significant effect of foliar nitrogen fertilization on fruit properties is shown in the Table 3. The only treatment that increased average fruit weight as well as fresh fruit weight in both seasons concerning the untreated control trees was the treatment U_1. Moreover, in comparison with the control trees, the U_1 treatment increased the fruit pulp/pit ratio by 1.39 and 1.42 in the first and second years, respectively. A regular pattern variation was not found for pit weight when comparing with the control in the two years (Table 3).

Macro and micro elements of leaves

Table 4 summarizes the leaf macro element (N, P, and K %) and micro elements (B and Fe mg/Kg) of each treatment at both sampling time during growth seasons, i.e., August and October. In August of the first year, the maximum amount of N and K were accompanied by

Table 3
The effect of Urea and nano-Nitrogen foliar applications on fruit traits of olive tree

Treatment	Fruit weight (gr)		Pulp weight (gr)		Pit weight (gr)		Pulp/Pit ratio	
	2017	2018	2017	2018	2017	2018	2017	2018
*Control	25.48^{d}	28.46^{d}	18.87^{d}	21.17^{d}	6.61^{d}	7.29^{ab}	2.85^{cd}	2.90^{c}
U_1	39.67^{a}	43.38^{a}	31.72^{a}	34.91^{a}	7.95^{bc}	8.47^{ab}	3.98^{a}	4.12^{a}
U_2	35.60^{b}	39.81^{b}	26.91^{b}	30.99^{b}	8.69^{ab}	8.82^{a}	3.09^{bc}	3.51^{bc}
nano-N_1	33.45^{bc}	38.62^{b}	25.85^{b}	30.52^{b}	7.60^{c}	8.10^{ab}	3.40^{b}	3.76^{ab}
nano-N_2	33.21^{c}	34.00^{c}	23.72^{c}	25.97^{c}	9.49^{a}	8.03^{ab}	2.49^{d}	3.23^{b}

* Control, (U_1 & nano-N_1), and (U_2 & nano-N_2) are 0, 1.02, and 1.36 g L^{-1} pure nitrogen, respectively. Means with the same letter in each column were not significantly different using Least Significant Difference test at $p \leq 0.01$.

Table 4
The effect of Urea and nano-Nitrogen foliar applications on leaf mineral composition of olive tree

Year (2017)										
Treatment	N %		P %		K %		B (mg/kg)		Fe (mg/kg)	
	August	October	August	October	August	October	August	October	August	October
*Control	1.40^{c}	1.51^{c}	0.13^{b}	0.14^{a}	0.99^{c}	1.10^{b}	16.00^{c}	22.00^{c}	105.00^{c}	113.00^{e}
U_1	1.65^{a}	1.66^{b}	0.17^{b}	0.14^{a}	1.35^{a}	1.35^{a}	29.33^{b}	36.00^{b}	142.66^{a}	150.00^{b}
U_2	1.51^{b}	1.84^{a}	0.26^{a}	0.13^{a}	0.69^{d}	1.28^{a}	33.33^{a}	39.33^{a}	146.00^{a}	160.07^{a}
nano-N_1	1.46^{bc}	1.56^{c}	0.17^{b}	0.14^{a}	1.19^{b}	1.29^{a}	27.33^{b}	33.66^{b}	121.33^{b}	147.00^{c}
nano-N_2	1.43^{c}	1.67^{b}	0.15^{b}	0.13^{a}	0.95^{c}	1.31^{a}	26.33^{b}	35.61^{b}	120.66^{b}	140.00^{d}
Year (2018)										
Treatment	N %		P %		K %		B (mg/kg)		Fe (mg/kg)	
	August	October	August	October	August	October	August	October	August	October
Control	1.47^{b}	1.55^{b}	0.09^{b}	0.15^{a}	1.26^{b}	1.32^{b}	17.00^{e}	25.00^{e}	111.66^{e}	116.00^{d}
U_1	2.12^{a}	2.13^{a}	0.13^{a}	0.15^{a}	1.50^{a}	1.52^{a}	37.33^{c}	37.90^{d}	146.33^{b}	151.33^{b}
U_2	2.03^{a}	2.12^{a}	0.13^{a}	0.13^{a}	1.54^{a}	1.62^{a}	41.00^{b}	42.66^{c}	161.33^{a}	164.33^{a}
nano-N_1	1.95^{a}	2.00^{a}	0.15^{a}	0.15^{a}	1.50^{a}	1.54^{a}	28.00^{d}	47.66^{b}	132.33^{c}	151.33^{b}
nano-N_2	2.03^{a}	2.04^{a}	0.13^{a}	0.15^{a}	1.51^{a}	1.62^{a}	56.33^{a}	57.03^{a}	125.00^{d}	153.59^{b}

* Control, (U1 & nano-N1), and (U2 & nano-N2) are 0, 1.02, and 1.36 g L−1 pure nitrogen, respectively. Means with the same letter in each column were not significantly different using Least Significant Difference test at $p \leq 0.01$.

treatment U_1. The P, B, and Fe mean values in the leaves increased significantly by the application of U_2. However, the Fe content did not show significant differences between U_1 and U_2 treatments. This pattern was repeated in the case of B and Fe content in October of the first year only with U_2 treatment; however, leaf P concentration in nitrogen treatments did not differ from the control. In October of the first year, the maximum amount of N in the leaves occurred as a result of applying the U_2 treatment and the minimum amount of K was recorded in control. In August and October of the second year, it was observed that foliar N fertilizers caused an increase in the leaf N, P, and K concentrations when compared with the untreated control trees, with the exception of P content in October of the second year which showed no significant difference. As a result, leaf B and Fe contents in August and October were significantly higher in nano-N_2 and U_2 treatments, respectively.

Chlorophyll and carbohydrate concentrations

The total chlorophyll content of olive leaves is presented in table 5. These content were varying between treatments. Among all treatments, the highest chlorophyll value obtained under U_1 spray in August and October first year. However, in the second year did not show significant difference between U_1 and U_2 treatments. The lowest value of chlorophyll was recorded in the untreated control trees in both seasons.

Table 5
The effect of Urea and nano-Nitrogen foliar applications on chlorophyll and carbohydrate contents of olive tree

Treatment	Year (2017)			
	Chlorophyll content (mg g^{-1} FW)		Carbohydrate contents (mg g^{-1} DW)	
	August	October	August	October
*Control	1.04[e]	0.94[d]	25.28[d]	30.16[d]
U_1	2.62[a]	2.51[a]	31.76[c]	45.29[c]
U_2	2.32[b]	2.25[b]	31.90[c]	54.97[b]
nano-N_1	1.86[d]	1.71[c]	38.10[b]	55.00[b]
nano-N_2	1.98[c]	1.77[c]	49.93[a]	57.10[a]
	Year (2018)			
Treatment	Chlorophyll content (mg g^{-1} FW)		Carbohydrate contents (mg g^{-1} DW)	
	August	October	August	October
Control	1.25[c]	1.12[c]	33.00[e]	38.78[e]
U_1	2.67[a]	2.56[a]	45.18[d]	55.03[d]
U_2	2.55[a]	2.53[a]	50.20[c]	56.90[c]
nano-N_1	2.01[b]	1.90[b]	54.60[b]	58.30[b]
nano-N_2	2.07[b]	2.02[b]	60.12[a]	61.73[a]

* Control, (U_1 & nano-N_1), and (U_2 & nano-N_2) are 0, 1.02 and 1.36 g L^{-1} pure nitrogen, respectively. Means with the same letter in each column were not significantly different using Least Significant Difference test at $p \leq 0.01$.

Carbohydrate value improved in trees receiving nitrogen treatments as compared with the control trees during the two years. During the first year, the carbohydrate concentration in the leaves in August and October were 1.97 and 1.89 fold higher in the nano-N_2 than the control trees. This pattern was 1.82 and 1.59 fold in the second year.

Discussion

Results of cumulative data from two years showed that nitrogen treatments led to an increase in the number of perfect flowers in comparison with control trees. This observation could be due to improvements in the nutritional status and availability of assimilates for flower organs development by the nitrogen application during the bud-swelling stage and before blooming. This confirms the fact that the period of differentiation before bloom is important for the development of perfect flowers and the application of foliar sprays during this time can be substantially effective (Bouranis et al*.,* 2001). In other words, an increase in the number of perfect flower was probably due to greater amounts of absorbance and availability of nitrogen for the sustenance of biochemical reactions in plant cells especially by U_1 treatment. Etehadnejad and Aboutalebi (2014) reported that the improvement of nitrogen in flower prolongs the fertilization period through increasing the ovule life. It seems that urea due to rapid absorption can increase the plant's utilization efficiency of nutrients in comparison with slow-release nano-fertilizers. Support this result, the positive effect of urea foliar spray on producing a high number of perfect flower has been reported in olive (Talaie and Taheri, 2000), mango (Nafees et al*.,* 2013) and pomegranate (Etehadnejad and Aboutalebi, 2014).

There are negative relationship and competition between growing shoots and flowering in olive tree (Lavee, 2007). Because of the high flowering intensity, i.e., the high number of inflorescences and perfect flowers, the trees were expected to have less vegetative growth. However, the cumulative data obtained from two years of study showed the highest growth rate in response to U_1 among other treatments. The application of U_1 resulted in more balance between vegetative and reproductive growth. This could be in part due to the role of nitrogen in growing traits via its contribution to cell division and elongation, carbohydrate metabolism, sugar transport, and the synthesis of chlorophyll, as well as in the structure of proteins and nucleic acids (Barker and Pilbeam, 2007). These results indicate that probably after urea spray, nitrogen rapidly was absorbed and converted to assimilate as required for growth. Nafees et al. (2013) showed that balance in vegetative and reproductive growth could be achieved by maintaining optimum nitrogen status in mango plants. The availability of N was reported as one of the most important factors affecting plant growth and under N deficiency, shoot growth was negatively influenced (Desouky, 2009).

Fruit set is an important component of yield and it is often believed that improving this index may improve tree yield (Rosati et al., 2010). However, yields are not always increased with fruit set (Beya- marshall and Fichet, 2017; Larbi et al*.,* 2011). In this study, an increase in fruit set dose not led to increased fruit yields. Generally, those treatments with low flower load had a great amount of fruit set. It seems that trees with the highest number of inflorescence and number of perfect flower have the greatest fruiting potential. Supporting this, it was reported that fruit set not only depends on the competition between inflorescences but also on the potential fruiting of the tree (Lavee et al., 1996). Moreover, Cuevas et al. (1994) and Lavee et al. (1999) reported that fruit set increased when inflorescence and flower number were reduced and vice versa; however, the yield was higher on the abundantly flowering shoots. Increase in yield may be attributed to the effect of nitrogen on improving the nutritional status of trees and produce of assimilates such as carbohydrate which may lead to stimulate the higher perfect flower induction in trees treated with U_1. This finding is in agreement with previous studies that reported limited access to mineral nutrition leads to low yield and the nutritional status of fruit trees affects crop yield (Casero et al., 2004; Nestby et al., 2005). It is also recognized that supplementary foliar fertilization during crop growth can improve the mineral status of plants and increase the crop yield (Kolota and Osinska, 1999). Concerning the use of urea, increases in yield with urea fertilization have been previously reported on fruit trees (Hasani et al*.,* 2016; Mitre et al., 2012; Sarker and Rahim, 2013). A positive effect of the foliar application of nano-nitrogen and urea on fruit yield has been reported in pomegranate (Davarpanah et al*.,* 2017). This indicates that different plants have a different response to the same nano fertilizer.

In comparison among nitrogen treatments, the high amount of oil percentage was achieved in those treatments which resulted in low crop load, i.e., nano-N_2. This suggest a negative correlation between oil percentage and crop load and could be associated with reduced availability of nutrient resources affecting oil synthesis in high fruit load condition (Gucci et al*.,* 2007).

Fruit, pulp and pit weight as well as pulp to pit ratio are used as criteria for olive fruit quality and quantity assessments. Among them, pulp weight and pulp to pit ratio are important parameters for the consumer acceptance of table olives (Rejano et al., 2010). The foliar application of nitrogen before blooming and pit hardening stages improved fruit quality in part due to enhancing of the assimilate formation and translocation to fruits. Hence, this resulted in an increasing of the cells number per fruit via encouraging cell division and enlargement. It is previously reported that during fruit cell division, high amounts of carbohydrate and nitrogen in the fruitlet tissues are needed for rapid cell division (Xia et al*.,* 2009). In comparison with nano-nitrogen, the application of similar concentrations of nitrogen in the form of urea has more positive effects on the mentioned traits. In agreement with these results, previous research revealed the positive correlation between leaf nitrogen content and fruit traits after urea foliar spray (Etehadnejad and Aboutalebi, 2014; Hasani et al*.,* 2016). Also, Davarpanah et al*.* (2017) reported that in the pomegranate tree average fruit weight increased with urea and nano-nitrogen foliar spray.

Leaf mineral characterization is used to identify diagnosing tree nutritional status and is an important tool for determining future fertilization recommendations (Fernández-Escobar et al., 2009). In the current study the levels of the evaluated mineral elements were above the threshold limit, except for nitrogen and boron values in control. It has been suggested that nitrogen influences the uptake of other plant nutrients. Furthermore, an increasing of leaf nitrogen concentration, following application of nitrogen, has been reported in apple (Amiri et al., 2008), and pomegranate trees (Hasani et al., 2016). We noticed that urea application led to more increase in leaf mineral element contents than those achieved by nano-nitrogen especially in the first year. These results are consistent with previous reports by Davarpanah et al. (2017) who reported that foliar application of urea affected leaf concentration of N better than that of the nano-nitrogen. Also, Fernández-Escobar et al. (2011) reported that foliar application of urea was effective in increasing the nitrogen content of olive trees. It has been pointed out earlier that the absorption of urea by the leaves of most crops is greater and faster than that of inorganic nitrogen forms (Fritz, 1977). This phenomenon may be due to the effect of urea in rapid translocation, which produces low osmotic pressure in the tissues and following water penetration with the plant and mineral element uptake. However, in the second year, nano-fertilizers probably due to characteristics such as their slow release property, mainly delay the release of the nutrients and extend the period of fertilizer effect which in turn, leads to an improvement in mineral element concentration.

The foliar application with nitrogen treatments resulted in an increasing amount of chlorophyll content. The chlorophyll value probably reflects the metabolic behavior of each tree about different mineral element content of leaves resulted by application of nitrogen fertilizers. Chlorophyll content is commonly determined by the availability of nitrogen in the tree and nitrogen is an essential part for chlorophyll molecule build (Chen et al., 2011; Netto et al., 2005). Moreover, the involvement of other nutrient minerals such as potassium and iron in the synthesis of chlorophyll and maintenance of chloroplast structure and function has been postulated (Collins and Duke, 1981; Rout and Sahoo, 2015; Shireen et al., 2018). Altogether, one can explain that the improvement of chlorophyll content in treatments of U_1 and U_2 should result from an increase in mineral element contents. Also, a negative influence of nitrogen deficiency on chlorophyll content and photochemical efficiency has been reported previously (Boussadia et al., 2010). Thus, nitrogen deficiency, according to Table 3 was probably the reason for a low amount of chlorophyll content in leaf samples from control trees. Unlike the higher mineral element content, the leaf chlorophyll content decreased in October. These results show that pigments were not only affected by endogenous nutrient factor but also by environmental criteria such as light and temperature variation. Accordingly, the influence of various environmental factors such as temperature, photoperiod, water availability, and the specific stage of maturity on leaf chlorophyll contents has been shown (Chen et al., 2011; Lee et al., 2011).

The leaf carbohydrate contents showed an increasing trend in response to foliar application of nitrogen. This could be explained in part by the role of nitrogen in stimulated sugar biosynthesis and transport of photo-assimilates. Furthermore, increases in carbohydrate after N application can be attributed to the important roles of N in chloroplast structure, CO_2 assimilation, and activation of enzymes involved in photosynthesis, which lead to increases in photosynthesis and carbohydrate accumulation (Garhwal et al., 2014; Kumar et al., 2014; Ramezanian et al., 2009). Also, the increase in carbohydrate contents under nano-N_2 treatment in both years may be related to the reduced sink demands. These results are in agreement with previous findings where it was confirmed that flower formation and fruit development are exhaustive processes in energy consumption (De la Rosa et al., 2000). In fact, increases in total sugars of olive leaf are in line with other fruit species (Abd El-Rhman and Shadia, 2012; Ghosh and Chattopadhyay, 1999; Singh et al., 2005). Moreover, total sugar was improved after foliar application with 1.8 kg N/ha nano-nitrogen and 16.3 kg N/ha urea in pomegranate (Davarpanah et al., 2017).

Conclusion

Results showed that traits such as the number of perfect flowers, fruit yield, and vegetative growth improved by foliar application of nitrogen fertilizers especially with U_1. In spite of the competition relationship between vegetative and reproductive growths, using U_1 resulted in a relatively balanced behavior between them. The balance is achieved when vegetative vigor and fruit load are in equilibrium and consistent with high fruit quality. Under treatments with low flowering, olive trees exhibited relatively high fruit set. However, this increase did not lead to high fruit yield. Moreover, our results showed that heavy fruit load in U_1 treatment in the first year unimpaired the flowering and fruit yield of the following year. Several features of the fruit such as good size, average fruit weight, and a high pulp to pit ratio were affected by U_1 treatment. Photosynthetic pigments and carbohydrates content in the leaves were increased using nitrogen, in part due to improvement in leaf nutrient status. The results of this study clearly showed that foliar spraying of nitrogen increases the concentration of each of macro and micro nutrients in the leaves especially during the first year. Generally, fertilizing the trees with urea was better than that with nano-nitrogen. However, nano-fertilizers are very innovative. Thus, more study is required especially in association with their contribution in olive oil yield and quality parameters.

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