Nano-Chelated Nitrogen Fertilizer as a New Replacement for Urea to Improve Olive Oil Quality

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ABSTRACT

Different sources of nitrogen fertilizers are being used in olive orchards, of which urea is widely used by olive growers. However, nano-chelated nitrogen (nano-N) is a newly found feature of the fertilizer with very little known information. In the current research, the impact of foliar spray of two nitrogen sources; urea (U) and nano-N on oil content and quality of olive cv. ‘Zard’ during two consecutive seasons were investigated. The olive trees were sprayed with 2.21 g (U1) and 2.95 g (U2), and 6 g nano-N1 and 8 g nano-N2 at several phenological stages of olive tree. The detailed observations showed that U1 significantly increased fruit yield. Monounsaturated fatty acid and the ratio of oleic acid to linoleic acid were increased by the nitrogen treatments, especially with nano-N2, whereas it decreased in the case of saturated fatty acid and polyunsaturated fatty acids. The application of both fertilizer sources improved the leaf mineral compositions as well as the oil quality such as free fatty acids, peroxide activity, K232 and K270 extinction coefficients, the chlorophyll and carotenoid pigments. Total phenolic content of the oil in olive trees sprayed with urea was lower than those treated with nano-N. In contrast, the oil antioxidant capacity was high in those trees treated with nano-N. Overall, the results showed that nano-fertilizer, especially nan-N2 treatment rather than urea, is an effective approach to improve oil quality.

Introduction

Olive (Olea europaea) is an important horticultural crop grown widely in Mediterranean region. The global demand for olive oil consumption is raising due to its nutritional, excellent sensory, medicinal, and therapeutic characteristics. The nutritional value of “virgin olive oil” is mostly attributed to fatty acids and many other metabolites including phenolic compounds, sugars and chlorophyll content (Franco et al., 2014). It has been shown that diets containing olive oil

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reduce cancer rates, cardiovascular diseases and oxidative stress (Knoops et al., 2004).

The oil quality and beneficial properties are influenced by several factors such as cultivar type, agro-climatic conditions and orchard management techniques (Tura et al., 2007). In orchard management, the nutritional status of olive tree as well as its oil quantity and quality properties depends on the amount and type of the fertilizers which are used for better growth and yield (Sayyad-amin et al., 2015; Bouhafa et al., 2014).

Among the mineral elements, nitrogen is of immense important for both tree growth and improving characteristics of fruits and oil as, the oil constituent requires both nitrogen and carbon sources (Patil et al., 1996). There are different sources of nitrogen fertilizers for provision of the nitrogen requirement of the plant. Urea, as a commercially available nitrogen fertilizer, is frequently used in agricultural fields (Etehadnejad and Aboutalebi, 2014). However, urea fertilizers usually cause problems (such as leaf necrosis and toxicity) that may override their advantages associated with crop productivity (Wijesinghe and Weerasinghe, 2015).

Reducing or eliminating the use of chemical fertilizers for the crops has attracted lots of attentions in recent years (Vishwakarma et al., 2018). Nano-fertilizers provide less harmful but more effective agricultural inputs (Wu et al., 2018). The latest researches have reportedly focused on the potential of nanotechnology for the nutritional value, quality and safety of food production (Thiruvengadam et al., 2018). Advancement in nano-science has improved the introduction of fertilizer formulations for better penetration into the cell and higher efficiency of nutrient uptake (Suppan, 2013). These fertilizers, as alternatives to the commonly used ones, can release their nutrients at slower and controlled rate making them more efficient for the aim of decreasing soil contamination (Khan and Fatima, 2017). Research undertaken on nano-fertilizers usage on some fruit trees species demonstrated their potential roles in improving crop yield and fruit physical and chemical properties (Zagzog and Gad 2017). It has been reported that the content of mineral elements in the leaves as well as fruit yield of pomegranate trees were improved by nanofertilizers such as boron, nitrogen and calcium (Davarpanah et al., 2016, 2017, 2018). The beneficial effects of nano-boron fertilizer foliar spray on the oil quality (including total phenolic content and antioxidant capacity) of olive has been reported previously (Rohi Vishekaii et al., 2019). However, to the best of our knowledge, no studies have been conducted to compare the effects of nanochelated nitrogen and common nitrogen fertilizer sources on olive oil quality attributes. This research was planned to compare the effects of urea and nano-nitrogen fertilizer on fruit yield and quality attributes of olive oil.

Material and Methods

Plant material and location of the study

This study was carried out during the 2017-2018 in a commercial olive orchard using olive cv. ‘Zard’, located in Guilan province of Iran (Manjil 49° 25′ E, 36° 44′ N, altitude 396 m). The orchard was planted on a 6 × 8 m² area in 2002. The soil had clay-loam texture, with a pH of 7.39 and an EC of 1.03 dS m⁻¹ in saturated extract. Trees were irrigated by a drip irrigation system.

Two sources of nitrogen fertilizers including urea (U) with 2.21 and 2.95 g nitrogen L⁻¹ and nano-chelated nitrogen (nano-N) with 6 and 8 g nitrogen L⁻¹, corresponding to 1.02 g (U₁ and nano-N₁) and 1.36 g (U₂ and nano-N₂) pure nitrogen per liter, respectively. The fertilizers were applied during four phenological stages including bud-swelling, before blooming, pit hardening of growing fruit (eight weeks after full blooming) and one week after common fruit harvest time for table olive. Each tree was sprayed with a total of five liters of fertilizers at each stage and
spraying with water was considered as control. To prevent probable fertilizer drops penetration into the soil, a polyethylene film was spread on the soil surface around the tree.

Nano-N fertilizer was obtained from Sodour Ahrar Shargh Knowledge-based Company (Khazra), Teheran, Iran (http://en.khazra.ir). The nano-chelated nitrogen fertilizer used in this study is designed and synthesized based on “Chelate Compounds” technology patented in the United States Patent and Trademark Office (USPTO) (Nazaran, 2012). In brief, the fertilizer is produced using a self-assembly method (bottom-up) applied in the production of materials. This method is an engineered approach according to which a nitrogen source is protected by an organic acid as a chelate agent, following that the organic acid polymerization leads to nano spheres production and then polymerization is stopped at a particular stage under specific and controlled conditions and finally the desired molecular size and dimension is obtained. The TEM image concerns the obtained nano spheres (approximately 30 nm) using this synthesis method. The distribution of the particles shows that the product contains homogeneous nano chelated nitrogen.

**Fig. 1.** Transmission electron microscopy (TEM) micrograph of nano chelated nitrogen used in the present study

**Determination of nutrient elements**

The content of macro and micro-nutrients of leaves were measured in August (during fruit pit hardening). N, boron and phosphorus (B and P), potassium (K), and iron (Fe) were respectively determined using Kjeldahl, colorimetric, flame emission spectrometry and atomic absorption spectrophotometry methods (Walinga et al., 1995).

**Tree yield and fruit yield efficiency**

At the green ripening stage fruits were collected from each replicate and weighed to determine fruit yield. Crop yield efficiency was achieved by dividing yield to trunk cross-section area in each tree (Rosati et al., 2017).

**Quantity and quality analysis of oil**

The oil content was determined in fruit’s pulp using the standard Soxhelt method with Hexane at 60-80 °C boiling point, and the results were expressed in the percentage of dry pulp weight (Avidan et al., 1997). Acidity (% of oleic acid), peroxide value (meq O₂ kg⁻¹), and UV absorbance (K₂₃₂ and K₂₇₀) were analyzed according to EEC 2568/91 (1991). The chlorophyll and carotenoid contents of oil were evaluated at 670 and 470 nm, respectively using spectrophotometer (Isabel Minguez-Mosquera et al., 1991). Total phenolic content (TPC) was determined using a spectrophotometer (UV-160A, Shimadzu, Japan), and the results were expressed as mg
gallic acid equivalent to 1 kg of oil (Singleton and Rossi, 1965). Antioxidant capacity was estimated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and expressed as percent DPPH inhibition value (Parisi et al., 2009). The fatty acids were measured by gas chromatography (7890 A, Agilent Technologies, Wilmington, DE, USA) described by Abbasi et al. (2008).

**Statistical analysis**

The trial was carried out based on a randomized complete block design (RCBD) with five treatments and three replicates. It included one tree per plot. Data were statistically tested using a two-way ANOVA, according to a combined analysis of variance using SAS (Statistical Analysis System; SAS Institute Inc., Cary, NC, U.S.A.) software.

Table 1. Soil analysis results of the experiment location (Olive orchard)

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Soil texture</th>
<th>pH</th>
<th>EC (dS m⁻¹)</th>
<th>Organic carbon (%)</th>
<th>Nitrogen (%)</th>
<th>Phosphorus (*Ava) ppm</th>
<th>Potassium (Ava) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30</td>
<td>Clay-loam</td>
<td>7.12</td>
<td>2.55</td>
<td>1.48</td>
<td>0.11</td>
<td>15.8</td>
<td>105</td>
</tr>
<tr>
<td>30-60</td>
<td>Clay-loam</td>
<td>7.16</td>
<td>1.78</td>
<td>1.29</td>
<td>0.14</td>
<td>18.50</td>
<td>130</td>
</tr>
</tbody>
</table>

*Ava: Available

Table 2. Effect of Urea (U) and nano-Nitrogen (nano-N) foliar applications on the leaf mineral composition of olive tree. Data represent the two-year average (2017 and 2018).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N %</th>
<th>P %</th>
<th>K %</th>
<th>B (mg/kg)</th>
<th>Fe (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.44±0.02</td>
<td>0.11±0.01</td>
<td>1.12±0.08</td>
<td>16.50±0.84</td>
<td>108.33±1.59</td>
</tr>
<tr>
<td>U₁</td>
<td>1.88±0.10</td>
<td>0.15±0.00</td>
<td>1.42±0.03</td>
<td>33.33±1.87</td>
<td>144.49±0.92</td>
</tr>
<tr>
<td>U₂</td>
<td>1.77±0.11</td>
<td>0.19±0.03</td>
<td>1.11±0.19</td>
<td>37.16±1.83</td>
<td>153.66±3.68</td>
</tr>
<tr>
<td>nano-N₁</td>
<td>1.70±0.11</td>
<td>0.16±0.01</td>
<td>1.34±0.07</td>
<td>27.66±0.44</td>
<td>126.83±2.67</td>
</tr>
<tr>
<td>nano-N₂</td>
<td>1.73±0.14</td>
<td>0.14±0.00</td>
<td>1.23±0.12</td>
<td>41.33±2.73</td>
<td>122.83±1.27</td>
</tr>
<tr>
<td>LSD (p ≤ 0.01)</td>
<td>0.15</td>
<td>0.06</td>
<td>0.13</td>
<td>2.79</td>
<td>3.44</td>
</tr>
<tr>
<td>Year</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Treatment</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

*Control, (U₁ & nano-N₁) and (U₂ & nano-N₂) contain 0, 1.02 and 1.36 g L⁻¹ pure nitrogen, respectively. Values are reported as mean ±SE of three replicates. *, ** and NS are significant at p ≤ 0.05, p ≤ 0.01, and not significant, respectively.

**Fruit yield and oil content**

Effect of urea and nano-N foliar applications on the yield and oil percentage is shown in Table 3. Olive tree yield and fruit yield efficiency were improved in response to fertilizers, and the highest value was achieved when 2.21 g urea (U₁) was sprayed (Table 3).

The results also showed that fruit oil percentage was significantly affected by nitrogen source and varied from 45.5% - 55.8% at U₁ and nano-N₂, respectively (Table 3).
Table 3. Effect of Urea (U) and nano-Nitrogen (nano-N) foliar applications on the yield and oil content of olive tree. Data represent the two-year average (2017 and 2018)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Yield (kg/tree)</th>
<th>Yield efficiency (kg/cm²)</th>
<th>Olive oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.50±2.50</td>
<td>0.08±0.01</td>
<td>51.18±0.26</td>
</tr>
<tr>
<td>U1</td>
<td>33.70±0.68</td>
<td>0.13±0.00</td>
<td>45.55±0.88</td>
</tr>
<tr>
<td>U2</td>
<td>25.03±1.33</td>
<td>0.11±0.00</td>
<td>51.04±0.36</td>
</tr>
<tr>
<td>nano-N1</td>
<td>22.30±1.26</td>
<td>0.09±0.00</td>
<td>52.90±0.11</td>
</tr>
<tr>
<td>nano-N2</td>
<td>23.00±1.35</td>
<td>0.09±0.00</td>
<td>55.82±1.35</td>
</tr>
<tr>
<td>LSD (p ≤ 0.01)</td>
<td>0.87</td>
<td>0.00</td>
<td>0.60</td>
</tr>
<tr>
<td>Year</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Treatment</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Year × Treatment</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Values are reported as mean ± SE of three replicates. ** and NS are significant at p ≤ 0.01.

Table 4. Effect of Urea (U) and nano-nitrogen (nano-N) foliar applications on the quality of olive oil. Data represent the two-year average (2017 and 2018)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Free fatty acid (%)</th>
<th>Peroxide value (meq O₂ kg⁻¹)</th>
<th>K₉₀ value</th>
<th>K₂₃₂ value</th>
<th>Chlorophyll (mg kg⁻¹)</th>
<th>Carotenoid (mg kg⁻¹)</th>
<th>Total phenolic content (mg kg⁻¹)</th>
<th>Antioxidant capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.41±0.03</td>
<td>10.22±0.11</td>
<td>1.07±0.08</td>
<td>0.12±0.01</td>
<td>1.55±0.25</td>
<td>0.97±0.22</td>
<td>48.63±0.55</td>
<td>28.94±1.37</td>
</tr>
<tr>
<td>U1</td>
<td>0.31±0.01</td>
<td>9.16±0.21</td>
<td>0.83±0.08</td>
<td>0.10±0.01</td>
<td>2.01±0.13</td>
<td>1.16±0.02</td>
<td>46.99±0.17</td>
<td>30.11±1.48</td>
</tr>
<tr>
<td>U2</td>
<td>0.14±0.01</td>
<td>8.38±0.31</td>
<td>0.74±0.09</td>
<td>0.11±0.01</td>
<td>2.19±0.17</td>
<td>1.42±0.11</td>
<td>48.40±0.46</td>
<td>31.29±1.75</td>
</tr>
<tr>
<td>nano-N1</td>
<td>0.25±0.01</td>
<td>9.13±0.22</td>
<td>0.79±0.10</td>
<td>0.11±0.01</td>
<td>1.92±0.15</td>
<td>1.11±0.05</td>
<td>55.49±0.88</td>
<td>36.70±3.25</td>
</tr>
<tr>
<td>nano-N2</td>
<td>0.14±0.01</td>
<td>9.15±0.26</td>
<td>0.58±0.11</td>
<td>0.06±0.01</td>
<td>2.06±0.14</td>
<td>1.11±0.01</td>
<td>60.65±0.92</td>
<td>41.72±5.05</td>
</tr>
<tr>
<td>LSD (p ≤ 0.01)</td>
<td>0.05</td>
<td>0.76</td>
<td>0.05</td>
<td>0.02</td>
<td>0.08</td>
<td>0.05</td>
<td>0.57</td>
<td>0.44</td>
</tr>
<tr>
<td>Year</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>**</td>
</tr>
<tr>
<td>Treatment</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Year × Treatment</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
</tr>
</tbody>
</table>

Values are reported mean ± SE of three replicates. *, ** and NS are significant at p ≤ 0.05, p ≤ 0.01, and not significant, respectively.

Oil Quality Parameters

The quality indices of oil from treated olive trees are shown in Table 4. The oil free fatty acid content was reduced by nitrogen fertilizers. The least value for this parameter was obtained in oil with application of nano-N₂ and U₂. Peroxide value showed a significant response to U₂ and nano-N₁. The lowest K₂₃₂ and K₂₇₀ contents among treatments were found with nano-N₂. The chlorophyll and carotenoid pigments were increased in oil obtained from treated trees by nitrogen, especially U₂.

Total phenolic content and the antioxidant capacity of the oil

An increase in the TPC was found in nano-N₂ treatment, whereas U₁ foliar application decreased its value (Table 4).

There was a significant difference between nano-nitrogen and urea application on oil DPPH scavenging activity. The application of nano-N improved oil antioxidant capacity by 44.2% in comparison with control (Table 4).

Fatty Acid Composition

The results of oil fatty acid composition are summarized in Table 5. Nano-N₂ fertilizer resulted in an increase in the oleic acid and a decrease in the palmitic acid contents. The control trees showed the lowest amount of oleic acid and the highest amount of palmitic acid. Linoleic acid content was low with nano-N₂ as compared to other treatments. Oleic to linoleic acid and monounsaturated fatty acids (MUFA) to saturated fatty acids (SFA) ratios in nano-N₂ treatment were 10.9 and 4.80, respectively.
Table 5. Effect of Urea (U) and nano-nitrogen (nano-N) foliar applications on olive oil fatty acid composition. Data represent the two-year average (2017 and 2018)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Palmitic acid (%)</th>
<th>Stearic acid (%)</th>
<th>Oleic acid (%)</th>
<th>Linoleic acid (%)</th>
<th>Linolenic acid (%)</th>
<th>SFA</th>
<th>MUFA</th>
<th>Oleic-Linoleic ratio</th>
<th>MUFA/SFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.75±0.36</td>
<td>3.12±0.06</td>
<td>56.43±1.08</td>
<td>1.63±0.03</td>
<td>19.94±0.02</td>
<td>0.48±0.13</td>
<td>20.07±0.30</td>
<td>56.06±1.06</td>
<td>2.07±0.19</td>
</tr>
<tr>
<td>U&lt;sub&gt;1&lt;/sub&gt;</td>
<td>14.94±0.36</td>
<td>3.22±0.02</td>
<td>65.01±0.50</td>
<td>1.12±0.09</td>
<td>14.20±0.07</td>
<td>0.44±0.09</td>
<td>18.16±0.56</td>
<td>66.21±0.71</td>
<td>4.54±0.09</td>
</tr>
<tr>
<td>U&lt;sub&gt;2&lt;/sub&gt;</td>
<td>14.78±0.53</td>
<td>2.75±0.09</td>
<td>68.39±0.75</td>
<td>1.26±0.13</td>
<td>13.11±0.62</td>
<td>0.45±0.00</td>
<td>17.54±0.61</td>
<td>60.95±0.65</td>
<td>5.28±0.13</td>
</tr>
<tr>
<td>nano-N&lt;sub&gt;1&lt;/sub&gt;</td>
<td>14.98±0.53</td>
<td>3.11±0.21</td>
<td>62.79±0.25</td>
<td>1.24±0.03</td>
<td>14.45±0.23</td>
<td>0.39±0.07</td>
<td>17.61±0.74</td>
<td>64.02±0.33</td>
<td>4.34±0.09</td>
</tr>
<tr>
<td>nano-N&lt;sub&gt;2&lt;/sub&gt;</td>
<td>12.65±0.16</td>
<td>2.77±0.15</td>
<td>72.70±0.76</td>
<td>1.30±0.11</td>
<td>6.08±0.25</td>
<td>0.37±0.04</td>
<td>15.41±0.30</td>
<td>74.00±0.86</td>
<td>16.85±0.67</td>
</tr>
</tbody>
</table>

†† Control, (U<sub>1</sub> & nano-N<sub>1</sub>) and (U<sub>2</sub> & nano-N<sub>2</sub>) contain 0, 1.02 and 1.36 g. L<sup>-1</sup> pure nitrogen, respectively. Values are reported mean ±SE of three replicates. ** is significant at p ≤ 0.01.

**SFA**: saturated fatty acids, **MUFA**: monounsaturated fatty acids.

Discussion

The analysis of leaf minerals were used to identify nutritional status of tree in terms of being balanced, excessive or deficient, and considered to be an essential tool for future fertilization recommendations (Fernandez-Escobar et al. 2011). An increase in the nitrogen concentration of the leaf following the application of nitrogen has been reported in apple (Amiri et al. 2008) and pomegranate trees (Hasani et al. 2016). In the current study, treatment with urea led to a higher concentration of minerals in the leaf, when compared to nano-nitrogen treatments. The results are in agreement with the findings of Davarpanah et al. (2017), who showed that the effect of urea foliar application on the leaves’ nitrogen content was better than that of nano-nitrogen. Also, Fernandez-Escobar et al (2011) reported that foliar application of urea was beneficial for improving the nitrogen content of olive trees. This phenomenon may be due to the effect of urea in rapid translocation in comparison with nano fertilizer which mainly delays the release of the nutrients and extends the fertilizer effect period leading to lower increase in mineral element concentration.

Fruit yield was increased by applying fertilizers, with pronounced response in U<sub>1</sub>, in part, due to the nitrogen effect on enhancement of the tree nutrient. Positive effects of urea foliar application on fruit yield have also been reported in mango (Sarker and Rahim, 2013). Davarpanah et al. (2017) found an obvious influence of spraying with both nano-nitrogen and urea on pomegranate fruit yield. It seems that nitrogen fertilizer in its urea formula, especially at low concentration (U<sub>1</sub>), is more effective in increasing of olive fruit yield efficiency. An increase in the oil content in trials with low crop load, i.e., nano-N, was also observed. These results signify a negative association between crop load and oil percentage, and could be correlated with low availability of assimilates which influence oil synthesis in high fruit load condition (Gucci et al., 2007). However, fruit oil content did not improve in the control trees, even with low crop load, which indicates that nano fertilizers have the potential for synchronizing the nutrients resources release with crop requirements. Bearing in mind the fact that nano-fertilizers can improve the reactivity of accompanying elements more than their bulk counterparts (Naderi and Danesh-Shahrazi, 2013), we assumed that foliar spray shortly after the fruit harvest for table olive might export the assimilation into the fruit to fulfill cell metabolism requirements for oil synthesis. Foliar application of nitrogen in improving of
the olive fruit oil content has also been reported previously (Elbadawy et al., 2016).

Free fatty acid content, a quality criterion for evaluating olive oil, showed a reducing trend among nitrogen treatments. The International oil council (IOC) standard allows a maximum of 0.8% for this index in extra virgin olive oil. Results showed that, regardless of their concentrations and / or formula, nitrogen treatments led to a reduction in the peroxide value compared to that of the control. The IOC standard for this index is ≤ 20 meq O₂ kg⁻¹ (Mailer and Beckingham, 2006). In general, when the peroxide value exceeds 10, it may means less stable oil (Mailer and Beckingham, 2006). The lowest value for K₂₃₂ and K₂₇₀ was observed in the nano-N₂ treatment. A direct relationship has been found between the oil quality and low amounts of K₂₃₂ and K₂₇₀ (Zegane et al., 2015). The K₂₃₂ index mainly refers to the early oxidation of oil, and is a symptom related to the polyunsaturated fatty acids. The K₂₇₀ is a symptom of carboxylic compounds in the oil and is demonstrated to the secondary oxidation products (Boskou et al., 1996). The oil obtained from all treated trees was of excellent quality attributes and agreed characteristics for extra virgin oil. Jordaño et al. (1990) found correlations between the mineral elements content and olive oil quality. So, apart from the various roles for nitrogen in plant physiological metabolism, the improvement of the oil chemical properties resulted from the effect of nitrogen fertilizer on the mineral content of leaf and more probably that of fruit. These results did not agree with those reported by Bouhafa et al., (2014) showing that foliar application of nitrogen did not improve the olive oil quality. Dag et al. (2018) reported the negative correlation of excess nitrogen with the acidity of olive oil. Such diversity in the results show that oil quality could depend on the amount of nitrogen, its application time as well as plant's own nitrogen level. No studies have so far dealt with the application of nano-chelated nitrogen to improve the quality attributes of olive oil.

Chlorophyll and carotenoid are among sensorial characteristics of the oil for consumer perception. These pigments were increased by utilization of nitrogen fertilizers in the current study. Nitrogen is a structural component of chlorophyll and has a major role in the pigments biosynthesis in the fruit. Our results were in agreement with the finding by Tekaya et al. (2013), who reported an enhancement of pigments in olive oil using urea foliar spraying.

The TPC, a relevant indicator of antioxidants capacity, is responsible for the protection of biological systems against oxidative stress. Also, it determines bitterness, flavor and fragrance of olive oil (Bendini et al., 2007). The nutritional status in the plant has exceptional role in the biosynthesis of TPC. Application of nitrogen in the form of urea not only failed to increase TPC in the oil, but decreased it as low as the control level. On the other hand, this parameter was improved by nano-N (nano-N₂ in particular). The foliar spray resulted in phenolic synthesis probably through the known mechanisms in nano fertilizers; including targeted delivery, chemical properties, and precisely distributed nutrients in responding to crop needs and environmental stimuli (Tarañdar et al., 2015). Beside nitrogen effect on biochemical parameters of olive oil, the influences of chelating reagents or other accompanying compounds of nano-chelate fertilizers should not be ignored (Nazaran, 2012). The antioxidant capacity of olive oil was determined by the ability of radical scavenging activity (Covas et al., 2006). Foliar application of nitrogen in the form of nano-N influenced the metabolism of antioxidant compounds and served as a factor to enrich the antioxidant capacity. However, it was decreased when the same amount of pure nitrogen was used in the form of urea. One probable reason may be that nutritional packaging elements in the form of
nano-chelate. Hence, it facilitates the absorption, translocation and releasing of assimilates due to their unique physicochemical characteristics. Also, oil from olive trees treated with nano-N had considerable high TPC, which in turn is the key factor for increasing antioxidant capacity (Fernández-Escobar et al., 2006). The enhanced accumulation of antioxidants compounds in plant tissues has been reported in response to the application of nano-materials (Corral-Diaz et al., 2014).

The fatty acid composition plays an important role in the quality of olive oil and its nutritional value. Oleic acid and palmitic acid amounts were characterized by reverse trend in response to nano-N2. Oleic acid is the most important monounsaturated fatty acid, and palmitic acid is the major undesirable saturated fatty acid in olive oil (Beltrán et al., 2005). It has been reported that nitrogen stimulates higher amounts of oleic acid in olive oil, and decreases the level of palmitic acid (Erel et al., 2013). Based on current study, these effects were remarkable with nano-N fertilizers rather than the urea. Moreover, the MUFA and the MUFA/SFA ratio, which are essential parameters in terms of oil oxidative stability and its nutritional value (Beltrán et al., 2005), were influenced more satisfactorily by nano-N2. In coordinate with that, a decrease in saturated fatty acids and an increase in the unsaturated fatty acids in response to foliar spray of nitrogen have been reported in cotton (Kheir et al., 1991). Linolenic and linoleic acids are generally known as the primary sources of polyunsaturated fatty acids in olive oil which tend to become unfavorable if their contents increase beyond a limit in olive oil (Mailer and Beckingham, 2006). The mean values of those mentioned compounds were at higher amounts in the control trees. Also, compared to the control, the oleic/linoleic ratio was improved by nitrogen fertilizers. This ratio was about 1.5 times with urea, and about 3.8 times with nano-N2 treatment more than that of the control. A positive correlation has been reported in terms of improved oil stability, the nutritional value and oleic/linoleic ratio in olive (Beltrán et al., 2004; Inglese and Gullo, 2000). The improvement of oil fatty acid composition by nitrogen treatments was accompanied by tree low crop load. The fatty acid indices in the control trees, with the lowest fruit yield, were not as high as treated trees by nitrogen treatments. Hence, one could suggest that fatty acid content in olive oil may also be affected by the nitrogen nutritional status and its assimilates.

**Conclusion**

We found that foliar spray with urea (2.21 g L−1) could increase olive fruit yield, but decreased its fruit oil content. The nano-N2 application improved oil quality attributes such as TPC and antioxidant capacity. Nano fertilizers influenced these parameters through steady releasing of N, synchronize release time with the mineral uptake pattern of plant as needed over an extended period, and also through facilitating nutrient transportation capability within the tissues. Meanwhile, the possible effects of chelating agents, an integral part of nano-fertilizers, should not be ignored. Based on the promising results, the application of nitrogen, particularly in the form of nano-nitrogen, should be included in orchard management approach that aims at producing high quality oils.

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Conflict of interest
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.

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