HortScience 52(2):288–294. 2017. doi: 10.21273/HORTSCI11248-16

Effects of Foliar Nano-nitrogen and Urea Fertilizers on the Physical and Chemical Properties of Pomegranate (*Punica granatum* cv. Ardestani) Fruits

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Additional index words. foliar fertilization, nanotechnology, urea, yield, fruit quality

***Abstract*. The aim of this study was to compare the effects of foliar fertilization with a nitrogen (N) fertilizer containing nanoparticles (nN) with those of foliar fertilization with urea on the characteristics of pomegranate fruits cv. Ardestani. The experiment was carried out using a completely randomized block design with five treatments and four replications (trees) per treatment during two consecutive years, 2014 and 2015. Two foliar applications of nN (0.25 and 0.50 g N/L, equivalent to ≈1.3 and 2.7 g N/tree or 0.9 and 1.8 kg N/ha; nN1 and nN2, respectively) and urea (4.6 and 9.2 g N/L, equivalent to ≈24.4 and 48.8 g N/tree or 16.3 and 32.5 kg N/ha; U1 and U2, respectively) were applied at full bloom and 1 month later, and trees not treated with any N fertilizer were used as a control. Results show that foliar N fertilization increased fruit yield (by 17% to 44%) and number of fruits per tree (by 15% to 38%). The highest fruit yields (17.8 and 21.9 kg/tree) and number of fruits per tree (62.8 and 70.1/tree) were obtained with the treatment nN2 (1.8 kg N/ha), whereas the lowest fruit yields (12.4 and 16.2 kg/tree) and number of fruits per tree (45.5 and 55.3/tree) were recorded in the control trees. The treatments U1 and nN2 increased fruit length (the latter only in the second season), whereas the treatment U1 increased average fruit weight (10% to 11%). The treatment nN2 increased aril juice and total soluble solids (TSS) in both seasons and titratable acidity (TA) only in the first one, whereas the treatment U1 increased TSS in both seasons and aril juice and TA in the second season. Treatments nN2 and U1 also increased total sugars and decreased total anthocyanins. On the other hand, fruit diameter, fruit cracking, peel thickness, aril content, weight of 100 arils, juice pH, maturity index, antioxidant activity, and total phenolic compounds were unaffected by N fertilization. Nitrogen fertilization increased the leaf concentration of N, whereas the leaf concentrations of P, K, Mn, and Zn were unaffected. Results indicate that pomegranate fruit yield was improved similarly with two applications (at full bloom and one month later) of nN fertilizer at a rate of 1.8 kg N/ha and with two applications of urea at a rate of 16.3 kg N/ha. Furthermore, fruit quality was improved more with the nN fertilizer at a rate of 1.8 kg N/ha than with two applications of urea at a rate of 16.3 kg N/ha.**

Pomegranate (*Punica granatum* L., Punicaceae) is a tree native from central Asia regions, from Iran to the Himalayas in northern India, and is one of the oldest known fruit crops. Pomegranate is nowadays widely cultivated in commercial orchards in Iran, India, China, and Afghanistan, and also in many of the Mediterranean basin countries, including Turkey, Greece, Italy, France, Spain, and Portugal (Artés et al., 2000; Holland et al., 2009; Matthaiou et al., 2014; Pekmezci and Erkan, 2003). Pomegranate fruits have been traditionally eaten fresh or consumed in processed products such as juice, jams, anardana (dried arils), jellies, carbonated drinks, syrups, garnish, and deserts (Al-Maiman and Ahmad, 2002; Kays, 1999; Opara et al., 2009).

Plant nutrition is crucial for agriculture production and crop quality, and ≈40% to 60% of the total world food production depends on the application of fertilizers. The average yields of some cereal crops in the United States, including maize, rice, barley, and wheat, decreased without N fertilizer inputs by 41%, 37%, 19%, and 16%, respectively (Roberts, 2009). Regarding fruit crops, fertilization during fruit growth can improve harvest and postharvest quality (Roberts, 2009). Nitrogen plays important roles in plant growth and development as well as in fruit yield and quality, being required for chlorophyll and enzyme synthesis and constituting a component of proteins, metabolites, and nucleic acids (Barker and Pilbeam, 2007; Titus and Kang, 1982). Therefore, N deficiency leads to growth limitation in all plant organs, including roots, stems, leaves, flowers, and fruits (Barker and Pilbeam, 2007). Urea is the most commonly used N source for foliar fertilization (Etehadnejad and Aboutalebi, 2014; Fernández et al., 2013; Swietlik and Faust, 1984) due to several characteristics, including its high solubility in water, nonpolarity, rapid leaf absorption, and low phytotoxicity (Bondada et al., 2001; Etehadnejad and Aboutalebi, 2014; Yamada et al., 1965). Although foliar fertilization is not aimed to fully replace soil fertilization, it has been found that the effects of foliar applications of urea in spring are similar or even better than those of soil applications regarding improvements in fruit yield and quality (Blasberg, 1953; Fisher and Cook, 1950; Hasani et al., 2016).

The use of nanofertilizers is the most important application of nanotechnology in agriculture so far (Agrawal and Rathore, 2014; Naderi et al., 2011). Nanofertilizers are aimed to make nutrients more available to leaves, consequently increasing nutrient use efficiency (Suppan, 2013). Some characteristics of nanoparticles, including the large specific surface area, unique magnetic/optical properties, electronic states, and catalytic reactivity confer nanoparticles a better reactivity than the equivalent bulk materials (Agrawal and Rathore, 2014). Regarding N fertilizers, the application of nanotechnology can provide fertilizers that release N when crops need it, eventually leading to increases in N efficiency through decreases in N leaching and emissions and long-term incorporation by soil microorganisms (Naderi and Danesh-Shahraki, 2013; Suman et al., 2010). In previous studies, urea-loaded zeolite chips (Millán et al., 2008) and nanocomposites containing N (Jinghua, 2004) have been used to induce a slow N release and increase plant N uptake. Other materials being used for the same purpose include nutrient sources coated with thin polymer films and nutrients encapsulated inside nanoporous materials (Rai et al., 2012).

Since no studies have been carried out to evaluate the effect of nano N fertilizers on pomegranate trees so far, the purpose of this study was to assess the effects of foliar sprays with a nano N fertilizer and urea on the physical and chemical characteristics of pomegranate fruits. Pomegranate is one of the most important crops in Iran and is cultivated in many areas. The pomegranate sweet–sour cultivar used, 'Ardestani', is of Iranian origin, late ripening and with round shape, red peel and aril, and it is mostly eaten fresh.

Materials and Methods

Experimental site, plant materials, and treatments. The experiment was carried out in two seasons, 2014 and 2015, in a commercial pomegranate (*Punica granatum*) orchard. The orchard was located in the central part of the Razavi Khorasan province in northeastern Iran (Tous Dasht; lat. 35°1′24.33″ N, long. 58°50′19.61″ E, altitude 967 m), and the soil was a coarse-loamy over fragmental, mixed, thermic xeric Torriorthents (64% sand, 12% clay, and 24% silt), with a pH of 8.08 in water and an electrical conductivity of 9.4 $dS \cdot m^{-1}$. The region is arid, with 248 mm of annual mean precipitation and a mean annual temperature of 14.8 °C. Trees were 8-year-old with three trunks, ≈2.5–3 m in height. Trees were planted in regular rows and spaced at 3 × 5 m (667 trees/ha) and irrigated by a drip irrigation system. Trees were treated in winter with N–P–K fertilizers at 150:150:150 g/tree. The experiment was carried out based on completely randomized block design with five treatments and four replications per treatment (each replication consisted in a single tree). Trees used in the 2015 season were different from those used in the 2014 one.

Two sources of N were used, a "Nano-chelated fertilizer N" (250 g N/L; thereafter called nN; from Sepher Parmis, Teheran, Iran), and urea (from Shiraz Petrochemical Company, Shiraz, Iran). The nN fertilizer is NH_4NO_3 based and contains nanoparticles (composition patent-protected, average size 50 nm, range from 23 to 80 nm; http://www.sepehrparmis.com/fa_IR/Pages/Page/نـــانو). The nN fertilizer was used in spray applications at concentrations of 0.25 and 0.5 g N/L (nN1 and nN2, respectively; 0.5 g N/L is the dose recommended by the manufacturer) and urea was used at concentrations of 4.6 and 9.2 g N/L (equivalent to ≈1 and 2% urea in U1 and U2, respectively). Four trees were not treated with any N foliar fertilizer and used as a control. Therefore, the five treatments used were control, nN1, nN2, U1, and U2. Similar foliar urea doses (0.5% to 2%) have been recently used in foliar fertilization experiments in pomegranate trees (Hasani et al., 2016; Ramezanian et al., 2009). The fertilizer solution was prepared by diluting the nN commercial liquid product or commercial urea with underground water available in wells in the orchard. Trees were sprayed twice per season, the first at full bloom, on 12 May 2014, and 26 Apr. 2015, and the second 1 month after the first one, in all cases with 5.3 L per tree (until full foliage wetting; total doses of N applied with nano N and urea fertilizers were 1.325 or 2.65 g N/tree and 24.38 or 48.76 g N/tree, respectively). During the sprays, the soil surface below the tree was fully covered with plastic to avoid soil fertilization. Leaves were sampled from the middle part of fruiting shoots (100 leaves from the three trunks all around the canopy in each tree) on 11 Aug. in the first season and on 6 Aug. in the second season. Fruits were harvested on 22 Oct. in the first season and on 14 Oct. in the second season, with the harvest date being based on general fruit appearance and fruit chemical properties (see below).

Plant mineral analysis. The concentrations of macro- and micronutrients in pomegranate leaves were measured in the Iranian laboratory of Ferdowsi University of Mashhad in the first season and in the laboratory of the Estación Experimental de Aula Dei (EEAD-CSIC), Zaragoza, Spain, in the second season. In Iran, samples were prepared as in Chapman and Pratt (1961), and total N, P, K, and Ca were determined using Kjeldahl method, spectrophotometry, flame photometry, and complexometry, respectively; Fe, Zn, and Mn contents were measured using atomic absorption spectrophotometry (AAS). In the Spanish laboratory samples were digested using a microwave device and analyzed for Fe, Mn, Zn, and Ca using AAS (Carrasco-Gil et al., 2016).

Fruit physical properties. To determine fruit physical properties, four fruits were randomly selected from each tree replication, and fruit weight was measured using an electronic balance. Fruit diameter and length, fruit calyx diameter (the widest part of the neck), and peel thickness were measured by using a digital vernier gauge. To determine peel weight and aril content of each fruit, fruits were manually peeled and the weight of total arils and peel were measured. The weight of 100 arils was measured and the juice volume of 100 g arils, extracted gently by a manual extractor, was expressed in mL per 100 g arils. For all physical parameters four replications per treatment and year were carried out.

Fruit chemical properties. Titratable acidity was determined by the titration method (to pH 8.2 with 0.1 N NaOH), and results expressed as percentage of citric acid. Total soluble solids and juice pH were measured at room temperature using a digital refractometer and a digital pH meter, respectively. The TSS:TA ratio was expressed as maturity index. Four replications per treatment and year were carried out.

To determine total phenolic compounds contents in juice, the Folin–Ciocalteu reagent method was used (Singleton and Rossi, 1965). Four replications per treatment and year were carried out. The results were expressed as mg gallic acid equivalents per 100 g of fruit juice (mg GAE/100 g of juice; Tehranifar et al., 2010).

Antioxidant activity was determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Brand-Williams et al., 1995), as described in Davarpanah et al. (2016a). First, 100 µL of juice was diluted with 10 mL of methanol:water (6:4, v/v) (final ratio juice/diluted methanol 1:100), and the diluted juice was mixed with 2 mL of 0.1 mM DPPH in methanol. Then, the mixture was shaken and left in darkness and at room temperature for 15 min, and the A_{515} was measured using a spectrophotometer (Cecil Bio Quest, CE 2502, Cambridge, UK). The reaction mixture without DPPH was used for the background correction. The antioxidant activity was calculated using the following equation: antioxidant activity (%) = $[1 - (\mathrm{Abs}_{\mathrm{sample}}/\mathrm{Abs}_{\mathrm{control}})] \times 100$. Four replications per treatment and year were carried out.

Total anthocyanins were estimated by the pH differential method using two buffer systems: 25 mM potassium chloride buffer, pH 1.0, and 0.4 M Na acetate buffer, pH 4.5 (Giusti and Wrolstad, 2001). Samples were diluted with potassium chloride buffer until the A_{510} was within the linear range of the spectrophotometer (Cecil Bio Quest, CE 2502). The same dilution factor was later used to dilute the sample with the Na acetate buffer. Readings were performed at 510 and 700 nm in the two buffers after 15 min of incubation, four times per sample. Total anthocyanin content was calculated as $[(A \times MW \times DF \times 100)/MA]$, where $A = (A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}$, where MW is the molecular weight (449.2), DF is the dilution factor, and MA the molar absorptive coefficient of cyanidin-3-glucoside (26.900). Results were expressed as mg cyanidin-3-glucoside/100 g of juice (mg C3G/100 g of juice). Four replications per treatment and year were carried out.

Total soluble sugar contents in juice were determined using the anthrone reagent method (Dubois et al., 1951). A certain volume of juice was diluted with distilled water, and then 0.1 mL of the diluted juice was added to four mL of anthrone (150 mg pure anthrone in 100 mL of H_2SO_4 72%). The sample was heated for 10 min in a boiling water bath, and after cooling at room temperature A_{625} was determined using a spectrophotometer. The total sugar contents was calculated by using a glucose standard curve. Four replications per treatment and year were carried out.

The experimental design used was a randomized complete block design with four replications. Data were statistically evaluated by analysis of variance. Data were analyzed using SAS base 9 software. Means were compared using Duncan's multiple range test at the 5% level ($P = 0.05$).

Results

The mean N leaf concentrations in August in both seasons in the control trees were 1.75% to

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Received for publication 5 Aug. 2016. Accepted for publication 10 Nov. 2016.

Research supported by Ferdowsi University of Mashhad (code: 3/32199), the Spanish Ministry of Science and Competitiveness (MINECO grant AGL2013-31988, co-financed with the European Regional Development Fund) and the Aragón Government (group A03).

We appreciate the support provided by Ferdowsi University of Mashhad (code: 3/32199) to carry out this study. We thank to Marga Palancar and Carmen Lope (EEAD-CSIC), for mineral analysis, the Managing Director of the Tous Dasht orchard for support in conducting the experiments and Jesús Val (EEAD-CSIC) for useful comments.

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1.83% (Table 1). The mean leaf concentrations of P, K, and Ca in both seasons in the control trees were ≈0.1%, 0.9%, and 2.4%, respectively, whereas the mean leaf concentrations of Fe, Mn, and Zn in both seasons were ≈113, 74, and 14 mg·kg^{-1} DW, respectively (Table 1).

Foliar N fertilizers increased the leaf N concentration when compared with the untreated control trees, with the exception of the nN1 treatment, where the increases in leaf N were not statistically significant (Table 1). Leaf N concentrations in the treated trees were 2.13% to 2.04% (a 16% to 17% increase over the untreated controls) in the nN2 treatment, 2.25–2.13 N% (23% to 22% increase) in the U1 treatment, and 2.30–2.22 N% (26% to 27% increase) in the U2 treatment. The results also show that N foliar sprays did not affect significantly the leaf concentrations of P, K, Fe, Mn, and Zn, whereas the concentration of Ca was slightly decreased by the nN2 and U1 treatments only in the 2nd year (Table 1).

Foliar N fertilization significantly increased pomegranate fruit yield (by 17% to 44%, depending on the treatment) and also led to increases in the number of fruits per tree (by 15% to 38%) when compared with the untreated control trees (Table 2). Among of all treatments, the highest fruit yields (17.8 and 21.9 kg/tree) and numbers of fruits per tree (62.8 and 70.1 fruit/tree) were obtained with the treatment nN2, whereas the lowest values were recorded in the untreated control trees (Table 2). Treatments nN2 and U1 gave similar fruit yields, but the effects of the treatment nN2 on the number of fruits per tree were better (by 8% to 9%) than those of the treatment U1. The effects of the nN1 and U2 treatments were not as good as those of the treatments nN2 and U1. Foliar sprays with the highest urea concentration used (U2) consistently caused leaf burn in both seasons.

The only treatment that increased average fruit weight in both seasons with respect to the untreated control trees was the treatment U1, which had a low dose of urea fertilizer (increases of 10% to 11%; the differences between the U1 and nN2 treatments were not statistically significant). The treatments U1 (in both seasons) and nN2 (only in the second season) also increased fruit length, with increases being greater for U1 (8% to 11%) than for nN2 (8%), when compared with the fruits of untreated control trees (Table 2). Other physical properties such as fruit diameter (Table 2), fruit calyx diameter, fruit cracking (Table 3), and peel thickness (Table 4) were unaffected by the treatments.

The only foliar treatment that improved aril juice in both seasons was the highest dose of nN fertilizer (nN2; by 7% to 9%), whereas the treatment U1 significantly increased aril juice (by 7%) only in the second season (Table 4). Foliar fertilization did not cause significant effects in the aril and peel contents, the aril/peel ratio (Table 3) and the weight of 100 arils (Table 4) in the two seasons studied.

The treatments nN2 and U1 increased TSS in pomegranate juice in both seasons (by 8% to 12%, depending on the treatment; Table 4). The highest TSS values (18.3% and 18.6%) were obtained with the nN2 treatment, whereas the lowest (16.3% and 16.8%) was observed in the fruits of the untreated control trees. Regarding juice total acidity, some N treatments increased TA in pomegranate juice; nN2 in the first season and U1 in the second season led to increases in TA, by 7% and 9%, respectively (Table 4). On the other hand, the maturity index and juice pH were unaffected with any of the treatments (Table 5).

Treatments nN2 and U1 led to a 6% decrease in total anthocyanins in both seasons (Table 5). The highest total anthocyanin values (7.91 and 7.63 mg/100 g) in both seasons were obtained with nN1, values significantly higher than those observed with treatments nN2 and U1 (7.31–7.16 and 7.33–7.15 mg/100 g) (Table 5). On the other hand, the amount of total phenolic compounds was not significantly affected by N fertilization (Table 5).

Total sugars increased after foliar N fertilization with treatments nN2 and U1 (about by 8% to 9% and 10% to 11% in the first and second season, respectively, depending on the treatment). The highest total sugars in the first (15.61 g/100 g juice) and the

Table 1. Effects of foliar applications of nano-N (nN) and urea (U) fertilizers on pomegranate leaf mineral concentration in 2014 and 2015.

	N (%)		P (%)		K (%)		Ca (%)		Fe (mg·kg^{-1})		Mn (mg·kg^{-1})		Zn (mg·kg^{-1})	
Treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	1.83 c	1.75 c	0.10 a	0.11 a	0.94 a	0.85 a	2.14 a	2.66 a	108.3 a	118.0 a	76.0 a	72.7 a	15.7 a	12.5 a
nN1	1.99 bc	1.87 bc	0.10 a	0.11 a	0.97 a	0.90 a	2.25 a	2.54 ab	110.1 a	119.9 a	76.9 a	73.6 a	17.0 a	16.0 a
nN2	2.13 ab	2.04 ab	0.11 a	0.12 a	0.99 a	0.86 a	2.21 a	2.32 c	110.8 a	124.2 a	76.8 a	66.7 a	17.1 a	15.1 a
U1	2.25 a	2.13 a	0.11 a	0.12 a	1.00 a	0.93 a	2.17 a	2.49 b	110.4 a	122.2 a	77.3 a	65.2 a	17.2 a	15.3 a
U2	2.30 a	2.22 a	0.11 a	0.12 a	1.03 a	0.87 a	2.16 a	2.63 ab	109.6 a	122.4 a	76.1 a	70.2 a	17.1 a	13.9 a

The nN fertilizer was used at rates of 0.25 (nN1) and 0.50 (nN2) g N/L, and urea was used at rates of 4.60 (U1) and 9.20 (U2) g N/L, respectively. Means with the same letter in each column were not significantly different at $P = 0.05$.

Table 2. Effects of foliar applications of nano-N (nN) and urea (U) fertilizers on pomegranate fruit yield, number of fruits per tree, fruit weight, fruit length, and fruit diameter in 2014 and 2015.

	Yield (kg/tree)		Number of fruits (per tree)		Fruit wt (g)		Fruit length (mm)		Fruit diam (mm)	
Treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	12.4 c	16.2 c	45.5 d	55.3 c	272.5 b	293.0 b	76.1 b	79.5 b	74.7 a	77.5 a
nN1	14.6 b	18.9 b	53.0 c	64.5 b	275.1 b	293.1 b	76.8 b	78.0 b	74.8 a	80.0 a
nN2	17.8 a	21.9 a	62.8 a	70.1 a	283.4 ab	311.1 ab	79.7 b	85.6 a	78.5 a	82.4 a
U1	17.4 a	21.2 a	57.8 b	65.0 b	301.0 a	326.1 a	84.2 a	86.0 a	80.6 a	86.6 a
U2	15.2 b	19.1 b	54.3 bc	63.8 b	280.0 b	299.4 b	78.8 b	81.8 ab	77.9 a	81.5 a

The nN fertilizer was used at rates of 0.25 (nN1) and 0.50 (nN2) g N/L and Urea was used at rates of 4.60 (U1) and 9.20 (U2) g N/L, respectively. Means with the same letter in each column were not significantly different at $P = 0.05$.

Table 3. Effects of foliar applications of nano-N (nN) and urea (U) fertilizers on pomegranate fruit calyx diameter, fruit cracking, aril content, peel content, and aril/peel ratio in 2014 and 2015.

	Fruit calyx diam (mm)		Fruit cracking		Aril content (%)		Peel content (%)		Aril/Peel ratio	
Treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	19.8 a	20.6 a	2.8 a	4.0 a	54.0 a	58.3 a	46.0 a	41.7 a	1.17 a	1.40 a
nN1	21.5 a	20.7 a	2.8 a	4.0 a	57.0 a	58.5 a	43.0 a	41.5 a	1.32 a	1.41 a
nN2	19.9 a	20.3 a	3.0 a	4.5 a	58.0 a	59.4 a	42.0 a	40.6 a	1.38 a	1.46 a
U1	19.8 a	20.4 a	3.0 a	4.5 a	56.6 a	59.1 a	43.4 a	40.9 a	1.30 a	1.44 a
U2	21.5 a	20.2 a	3.0 a	4.0 a	57.9 a	58.6 a	42.1a	41.4 a	1.37 a	1.41 a

The nN fertilizer was used at rates of 0.25 (nN1) and 0.50 (nN2) g N/L, and Urea was used at rates of 4.60 (U1) and 9.20 (U2) g N/L, respectively. Means with the same letter in each column were not significantly different at $P = 0.05$.

second season (15.76 g/100 g juice) were obtained in trees treated with nN2 and U1, respectively, whereas the lowest ones (14.32 g/100 g juice and 14.18 g/100 g juice) in both seasons were recorded in the fruits of the untreated trees (Table 5). On the other hand, the antioxidant activity of pomegranate juice was not affected significantly by the N foliar treatments (Table 5).

Discussion

Leaf N concentrations found in untreated pomegranate trees were below 2%. Although no information on N sufficiency ranges in pomegranate are available to the best of our knowledge, leaf N concentrations lower that 2% are generally considered low in many fruit tree crops, including almond, apple, apricot, cherry, fig, lemon, and peach (Benton-Jones et al., 1991). Similar leaf N concentrations (in the range 1.73% to 1.94%) to those found here have been reported in Iranian pomegranate trees grown in the field without any fertilization (Hasani et al., 2012, 2016) or with a standard fertilizer management (Davarpanah et al., 2016a). In one of the main pomegranate crop areas in Turkey the N leaf concentrations in August were ≈2.2%, values higher than those found here (Korkmaz and Askin, 2015).

Foliar N treatments led to increases in the leaf N concentration up to values higher than 2%, with the exception of the more diluted nanofertilizer (nN1). Increases found in the concentration of N in pomegranate leaves after N fertilization are similar to those observed in grape (Delgado et al., 2006), apple (Amiri et al., 2008), and pomegranate trees (Hasani et al., 2016), and a positive linear correlation between the N concentrations applied and leaf N concentration has also been reported in pomegranate (Hasani et al., 2016). In most previous studies foliar N sprays led to increases in the concentration of N in the leaves, but it is known that the internal distribution of N absorbed after foliar fertilization depends on the timing of application. Nitrogen absorbed from urea sprays in June was mostly retained in leaves, whereas N sprays in autumn usually lead to a fast export from the fertilized leaves to perennial parts of the plant (Hill-Cottingham and Lloyd-Jones, 1975; Rosecrance et al., 1998; Shim et al., 1972; Swietlik and Faust, 1984; Tagliavini et al., 1998; Titus and Kang, 1982). For instance, when 1-year-old potted apple plants were foliar fertilized with labeled urea in autumn, up to 64% of absorbed leaf ^{15}N was translocated to other parts of the plant within 20 d (Dong et al., 2002).

Foliar N treatments improved fruit yield mainly due to increases in the number of fruits per tree, with the best results being obtained with the treatments supplying ≈2.7 and 24.4 g N/tree (nN1 and U1, respectively). Increases in yield and number of fruits per tree with N fertilization have been previously reported on citrus (El-Otmani et al., 2002; Lovatt, 1999), apple (Amiri et al., 2008), sweet cherry (Mitre et al., 2012), and mango (Sarker and Rahim, 2013), whereas in pomegranate trees it has been reported that soil urea application increased fruit yield and number of fruits per tree, with urea sprays being ineffective in increasing fruit yield (Hasani et al., 2016). Processing of N uptake by plant roots can be limited by soil low temperature and low activity of roots, whereas foliar N application is insensitive to those factors (Etehadnejad and Aboutalebi, 2014). The increases found in fruit set, number of fruits per tree and crop yield with foliar N fertilization can be attributed to the physiological and metabolic roles of N in flowering and fruit set, including supplying carbohydrates, which are necessary for flower bud growth, flower initiation and development, ovule lifespan, effective pollination, and fertility (Etehadnejad and Aboutalebi, 2014; Lovatt, 1994; Stiles, 1999).

Nitrogen fertilizer sprays also tended to improve fruit size and average weight, with treatment U1 leading to particularly large fruits, although a concomitant slight reduction in the number of fruits per tree led to fruit yields similar to that obtained with the treatment nN2. The increases found in fruit size are in line with data found in previous studies in pomegranate and other crops (Dadrasnia et al., 2009; Etehadnejad and Aboutalebi, 2014; Hasani et al., 2016; Mitre et al., 2012; Sánchez et al., 2007; Singh et al., 2005). In pomegranate, foliar applications of urea at concentrations of 1% and 2% at full bloom and 1 month after full bloom increased fruit length and diameter (Ramezanian et al., 2009). Improvements in fruit physical parameters after N application may be due to increases in the efficiency of metabolic processes, since N is a constituent of proteins, enzymes, and chlorophyll related to photosynthesis and growth. The developing fruit acts as a strong sink (Garhwal et al., 2014; Sharma et al., 2014), and it is well known that during fruit cell division, high amounts of C and N in the fruitlet tissues are needed for rapid cell division (Cheng et al., 2007; Xia et al., 2009). Also, N application improves leaf photosynthesis and increases N concentrations in fruit tissues needed for early fruit growth (Corelli Grappadelli et al., 1994, 2007). A low N supply has been shown to decrease the fruit N concentration and C supply to fruits, leading to a decrease in cell

Table 4. Effects of foliar applications of nano-N (nN) and urea (U) fertilizers on pomegranate weight of 100 arils, juice content, peel thickness, total soluble solids (TSS) and titratable acidity (TA) in 2014 and 2015.

	Wt of 100 arils (g)		Aril juice (mL 100/g arils)		Peel thickness (mm)		TSS (%)		TA (%)	
Treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	37.1 a	38.0 a	61.3 b	62.5 c	22.3 a	22.3 a	16.3 c	16.8 c	1.82 b	1.74 b
nN1	37.4 a	38.2 a	62.8 b	63.3 bc	21.1 a	22.1 a	17.2 bc	17.5 abc	1.84 b	1.84 ab
nN2	39.3 a	39.0 a	65.6 a	68.3 a	22.0 a	21.7 a	18.3 a	18.6 a	1.94 a	1.89 b
U1	38.1 a	39.0 a	63.6 ab	67.0 ab	21.6 a	21.9 a	17.7 ab	18.1 ab	1.84 b	1.90 a
U2	37.2 a	38.8 a	61.8 b	63.9 bc	21.5 a	21.5 a	17.0 bc	17.4 bc	1.83 b	1.84 ab

The nN fertilizer was used at rates of 0.25 (nN1) and 0.50 (nN2) g N/L, and Urea was used at rates of 4.60 (U1) and 9.20 (U2) g N/L, respectively. Means with the same letter in each column were not significantly different at $P = 0.05$.

Table 5. Effects of foliar applications of nano-N (nN) and urea (U) fertilizers on pomegranate maturity index, pH, total phenolic compounds, total anthocyanins, total sugars and antioxidant activity in 2014 and 2015.

	Maturity index (TSS:TA ratio)		pH		Total phenolic compounds (mg GAE 100/g juice)		Total anthocyanins (mg C3G 100/g juice)		Total sugars (g 100/g juice)		Antioxidant activity (%)	
treatment	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Control	8.95 a	9.65 a	3.51 a	3.68 a	409.13 a	405.05 a	7.76 ab	7.56 a	14.32 b	14.18 c	24.23 a	24.02 a
nN1	9.32 a	9.52 a	3.49 a	3.48 a	409.72 a	406.80 a	7.91 a	7.63 a	14.47 b	14.56 bc	24.40 a	25.01 a
nN2	9.41 a	9.84 a	3.39 a	3.43 a	409.66 a	406.70 a	7.31 c	7.16 b	15.61 a	15.54 ab	24.88 a	24.97 a
U1	9.62 a	9.52 a	3.48 a	3.42 a	408.37 a	405.62 a	7.33 c	7.15 b	15.52 a	15.76 a	25.17 a	24.45 a
U2	9.28 a	9.46 a	3.50 a	3.47 a	409.84 a	406.54 a	7.37 bc	7.37 ab	14.46 b	14.59 bc	24.84 a	24.87 a

TSS = total soluble solids; TA = titratable acidity; mg GAE 100/g juice = mg gallic acid equivalents per 100 g of fruit juice; mg C3G 100/g juice = mg cyanidin-3-glucoside/100 g.

Nano-N was used at rates of 0.25 (nN1) and 0.50 (nN2) g N/L, and Urea was used at rates of 4.60 (U1) and 9.20 (U2) g N/L, respectively. Means with the same letter in each column were not significantly different at $P = 0.05$.

division, whereas increases in the concentration of leaf N led to larger apple fruits through increases in photosynthesis and number of cells per fruit (Xia et al., 2009).

Pomegranate fruit juice content was increased with the treatment nN2 in both seasons and with the treatment U1 only in the 2nd season. In previous studies, foliar sprays of N increased fruit juice contents in citrus (Garhwal et al., 2014; Singh and Singh, 1981), although N application at high concentrations decreased mandarin fruit juice via increases in fruit peel thickness (Garhwal et al., 2014). Increases in mineral concentrations in cells after fertilization can increase turgor pressure and stimulate water absorption (Kumar et al., 2014).

Some changes in fruit chemical properties occurred on fertilization. Foliar N sprays increased juice TA in pomegranate fruits in some cases, although the effects were not consistent in the 2 years of the study. Increases in TA with N fertilization have been previously reported in pomegranate (Prasad and Mali, 2000) and jujube (Abd El-Rhman and Shadia, 2012). Conversely, it has been reported that urea sprays at full bloom stage reduced TA in pomegranate fruits, whereas foliar application of urea at 1 month after full bloom had no significant effects on this parameter (Ramezanian et al., 2009). Increased TA with N sprays can be due to increase in synthesis and translocation of organic acids in the fruits (Garhwal et al., 2014).

Increases in TSS in juice were found with the treatments nN2 and U1. This is in line with previous results in mango (Sarker and Rahim, 2013; Singh et al., 2005), jujube (Abd El-Rhman and Shadia, 2012), and persimmon (Choi et al., 2013). In pomegranate, soil application of N has been reported to increase significantly TSS (Hasani et al., 2016), whereas Prasad and Mali (2000) reported that N application had no significant effects on TSS. Increases in TSS after N application can be contributed 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 and also consequently increase in TSS (Garhwal et al., 2014; Kumar et al., 2014; Ramezanian et al., 2009; Stiles, 1999).

The treatments nN2 and U1 resulted in significant increases in total sugars, in line with previous studies with other fruit species (Abd El-Rhman and Shadia, 2012; Ghosh and Chattopadhyay, 1999; Singh et al., 2005). Nitrogen fertilization led to increased total, reducing and nonreducing sugars in pomegranate (Prasad and Mali, 2000), and total and reducing sugars in Guava fruits (Sharma et al., 2014). It has been reported that the effect of N fertilizers on sugar increase may help absorption of other mineral nutrients, improving fruit quality (Sharma et al., 2014).

Regarding anthocyanins, foliar sprays with the nN1 treatment did not have any effect on the total anthocyanin contents in comparison with the control treatment, whereas application of the treatments nN2, U1, and U2 led to decreases in this parameter. This is in line with results obtained in grape, where N application at moderate levels increased anthocyanin accumulation and high amounts of N fertilizers promoted vegetation growth and decreased polyphenols and berry color (Delgado et al., 2006). Although N is involved in anthocyanin formation (Saure, 1990), there are reports indicating that applications of N fertilizer had no effects on anthocyanin contents (Boonterm et al., 2011) and that excessive amounts of N fertilizers led to decreases in anthocyanins (Jeppsson, 2000). It has been reported that the N:K ratio is an important factor in concentrations of polyphenols and anthocyanins in grape (Delgado et al., 2006). Nitrogen deficiency leads to decreases in photosynthesis through decreases in chlorophyll and disruption of photosynthetic membranes, thus increasing sensitivity to high light levels, whereas anthocyanins act as a photoprotective pigments that may provide protection against light-induced oxidative damage (Guidi et al., 1998). Nitrogen stress can affect the expression of genes encoding enzymes associated with flavonoid and anthocyanin biosynthesis. Anthocyanin content increased 3.4-fold in tomato leaves as a result of N deficiency (Bongue-Bartelsman and Phillips, 1995).

Other fruit quality parameters, including aril content, weight of 100 arils, juice pH, maturity index, antioxidant activity, and total phenolic compounds were unaffected by the foliar N fertilizer treatments, although other studies have reported that foliar urea sprays increased aril content in pomegranate fruits (Hasani et al., 2016) and weight of 100 arils (Hasani et al., 2016; Ramezanian et al., 2009). The total phenolic compounds and antioxidant activity were unaffected with N fertilization, and this is in line with previous results in chili pepper (Núñez-Ramírez et al., 2011).

Conclusion

Results show that two of the four foliar N treatments used, nN at 2.7 g N/tree and urea at 24.4 g N/tree (equivalent to 1.8 and 16.3 kg N/ha, respectively) can improve leaf N content as well as fruit yield and quality of pomegranate cv. Ardestani grown in northeastern Iran. It is remarkable that a treatment with only 1.8 kg N/ha can provide beneficial effects on fruit yield and quality. This low N input is in line with good management strategies regarding preservation of the environment. The other two N treatments, nN at 1.3 g N/tree and urea at 48.8 g N/tree, do not appear to be usable in practice, the first because the lack of effects and the second because of the associated foliar damage (leaf burn). The effects of these N foliar fertilizers on fruit yield (consistently higher than 30% in the 2 years of study) can be mainly ascribed to the increase in the number of fruits per tree, whereas less strong effects in fruit quality, including increases in juice content, TSS, and total sugars and decreases in anthocyanins were also observed.

Results obtained indicate that a small amount of N applied via foliar fertilization improves yield and quality in pomegranate orchards established in nutrient poor soils in northeastern Iran. It has been recently shown that foliar fertilization with Zn and B (Davarpanah et al., 2016a) and Fe (Davarpanah et al., 2016b) also increase fruit yield and quality in pomegranate grown in the same area. The fact that foliar applications of urea are much less efficient that those of nN may suggest that pomegranate trees in the orchard cannot use efficiently urea applied via foliage. This could be due to a deficiency of Nickel (Ni), since the Ni concentration in pomegranate leaves was only $\approx 1\ \mu g \cdot g^{-1}$ DW (not shown). Nickel is a constituent of the urease enzyme, needed for urea degradation (Polacco et al., 2013). Therefore, further work should explore the possibility of using multielemental foliar spray treatments, combining N, Fe, Zn, B, and perhaps Ni. The use of multielemental foliar fertilizers is still poorly explored, but it has been recently shown that multielemental (Fe and Mn) foliar treatments can provide good results in tomato (Carrasco-Gil et al., 2016). On the other hand, it is not yet possible to explain mechanistically the mode of action of the nano N foliar fertilizer on pomegranate trees, since no detailed physiological experiments have been carried out and no comparable studies have been published in other crops.

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