

Foliar Nutrient Applications to Barberry (*Berberis vulgaris*). II: Effects on Leaf Nutrient Content and Physico-Chemical Characteristics of Fruit and Yield

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ABSTRACT

Barberry (*Berberis vulgaris* L.) is exclusively and widely cultivated in the South Khorasan province of Iran. This experiment aimed to evaluate the foliar application of urea, boric acid, iron sulfate, zinc sulfate, and copper sulfate effects on the nutrient content of leaf, yield, and physicochemical properties of barberry fruit. The experimental design was a randomized complete block as factorial with three replications. In the current research, urea with three levels (0, 1 and 2%), boric acid, iron sulfate, zinc sulfate and copper sulfate, each with three levels (0, 1500 and 2500 mg/L), and all of the fertilizers have been sprayed on barberry before full bloom at the rate of 3 L/tree. Concentrations of nitrogen, boron, iron and zinc in treated leaves of the trees were significantly higher than the untreated trees. A significant increase in yield was found by the application of 1% and 2% urea and foliar spraying all of the fertilizers together with all concentrations compared to the control. Also, the fruit weight was significantly enhanced using 1% and 2% urea, foliar spraying of all the fertilizers together (urea 2% + other fertilizers each 2500 mg/L) and iron sulfate 2500 mg/L compared to control. Foliar spraying of fertilizers (urea 2% + other fertilizers each 2500 mg/L) brought about the highest fruit weight (0.38 g). Urea, boric acid and foliar spraying of all the fertilizers with different concentrations significantly increased pH, total anthocyanin and total soluble solids and significantly decreased total acids in comparison with the control. It can be concluded that foliar application of urea with concentrations of 1% and 2% increased the content of leaf nitrogen, yield, TSS, total anthocyanin, and pH of barberry fruit.

INTRODUCTION

The barberry (*Berberis vulgaris* L.) belongs to the Berberidaceae family. The plant is resistant to drought and salinity; it has been cultivated widely in the southern Khorasan province of Iran [1]. Barberry fruit is consumed fresh or processed. The organs of this tree including root, skin, leaf, and fruit especially have medicinal properties due to their various alkaloids. In traditional medicine, fruits have been used to strengthen the liver, heart, digestion, relieve pain, and purify the blood. During recent years, the use of barberry fruit has increased in the food industry and various products are produced like drinks, jelly, pasta, jam, and food color [2]. Barberry fertilizers have not been researched in the context of Iran. During fruit growth, nutrition is a major factor affecting the

features of the fruit [3]. Optimizing the consumption of nutrients is one of the effective methods for increasing the yield of trees. Since much of Iran's soils are limestone, particularly areas of barberry cultivation, have a high production risk, due to the high pH and the shortage of low waste and organic matter, there is always a problem. In these soils, due to the presence of alkaline pH and high concentrations of calcium ions, some of the nutrients are inorganic and insoluble in plants which can be absorbed by pH. On the other hand, in calcareous soils, a large amount of bicarbonate ion has been produced, which reduces the absorption capacity of trace elements particularly iron while increasing the pH of the soil [4]. Leaf spraying is beneficial for improving trace element deficiency symptoms. Iron binds proteins to form important

enzymes in plants. Zinc is a component of many herbal enzymes and plays a role in plant processes, particularly in the synthesis of indole acetic acid hormone (IAA) [5]. Also, nitrogen interferes with the synthesis of enzymes and increases cell division and cell size [4]. Moreover, it increases the photosynthesis impact on the activity of enzymes that affect photosynthesis [3,6]. Nitrogen spraying in the spring enhances photosynthesis due to expanded leaf area [7]. Urea spraying has increased the fruit size and yield of the jujube [8,9]. Also, urea has increased the yield of other fruits including grape vines and almonds [7,10]. The addition of boric acid improved the fruit size and yield of the jujube [11]. In this research, we investigated the effect of foliar spraying of various concentrations of urea, boric acid, Iron sulfate, zinc sulfate and copper sulfate, both alone and in combination, on the tree mineral nutrition status and Physico-chemical characteristics of fruit and yield barberry. The study trees were grown in an important area of cultivation barberry in the east of Iran. In this area, soils are high in pH and low in organic matter and therefore poor in nutrients.

MATERIALS AND METHODS

The Experimental Site, Plant Materials and Treatments

This experiment was conducted at the barberry (*B. vulgaris*) Research Orchard of the Islamic Azad University of Birjand, Located at the latitude of 32° 52' N, longitude 59° 13' E, and elevation 1410 m above the sea level in two seasons, 2015 and 2016. The experiment was carried out as a randomized

complete block factorial design with three replications. The region is arid, with 170 mm of annual mean precipitation and a mean annual temperature of 16.5°C. Trees were planted in regular rows, with a 3 × 3m frame, irrigated by the drip irrigation system. Urea fertilizers with concentrations of 0, 1 and 2%, boric acid, iron sulfate, zinc sulfate and copper sulfate each with concentrations of 0, 1500 and 2500 mg/L alone and all the fertilizers together (urea + boric acid + iron sulfate + zinc sulfate + copper sulfate) with various concentrations (urea 0% + other fertilizers each zero mg/L; urea 1% + other fertilizers each 1500 mg/L; and urea 2% + other fertilizers each 2500 mg/L) were sprayed onto the barberry before full bloom (4 May, in two seasons) at the rate of 3 L/tree. All solutions were formulated in DI water with 0.50% adjuvant. Ethanol and hot DI water had been used to dissolve boric acid. Treatments were applied using a calibrated professional backpack sprayer. Replications included trees of the same age (16 years), vigor, and size. Spraying has been performed during the evening with cool air over the whole tree. The same procedure has been followed uniformly for all the trees. The fruits have been harvested in two seasons on 19 November.

Representative soil samples were collected at three different depths, sampling in a W pattern and the samples were analyzed following the procedures described by Gasparatos *et al.* [12]. The soil of the experimental orchard was loamy (40% sand, 10% clay and 50% silt), with a pH of 8.07 and EC of 2.97 dS/m. Some characteristics of the orchard soil in different depths are given in Table 1.

Table 1 Some physical and chemical properties of the soil profile in the experimental orchard

Parameters	Depth (cm)		
	0-30	30-60	60-100
Sand (%)	40	41	42
Silt (%)	50	49	49
Clay (%)	10	9	10
EC (dS/m)	2.97	2.05	1.67
pH	8.07	8.04	7.92
Organic matter (%)	0.34	0.17	0.09
N (%)	0.04	0.03	0.01
K (mg/Kg)	285	205	119
P (mg Kg)	6.5	2.5	2.1
Ca (cmolc/Kg)	39.8	38.2	37.4
Mg (cmolc/Kg)	6.82	5.3	5.1
Fe (mg/Kg)	2.24	2.01	1.99
Zn (mg/Kg)	0.51	0.45	0.39
Mn (mg/Kg)	4.07	3.85	2.25
Cu (mg/Kg)	2.99	2.01	1.5

Plant Measurements

The concentration of macro-and micro-elements in leaves

To determine leaf nutrient concentrations, samples of 70–80 fully expanded leaves per study tree were collected approximately two weeks after treatment (20 May). The samples were prepared according to Chapman and Pratt method [13], and total N, P, K and Ca were determined using the Kjeldahl method, spectrophotometry, flame emission photometry and complexometry, respectively; B was measured using azomethine H, and Fe, Zn and Mn concentrations were measured using atomic absorption spectrophotometry (AAS). These experiments were conducted in the laboratory of Islamic Azad University of Birjand.

Physical Characteristics

The weight of the fruits was measured in grams with a scale (accuracy of 10^{-4} gr). Fifty fruits have been randomly selected from each replicate and measured by their average weight. For measuring the dry weight, 15 grams of the barberry fruits were placed in an incubator at the temperature of 72°C for 48 hours. Dry weights were measured with a scale (accuracy of 10^{-4} gr). The yield of the trees was measured in kilogram with a scale (accuracy of 10^{-3} gr).

Chemical Haracteristics

Titrate acidity (TA) was determined by the titration method (to pH 8.2 with 0.1 N NaOH), and the results were expressed as the percentage of citric acid. Total soluble solids (TSS, as %) and juice pH were measured at room temperature using a digital refractometer and a digital pH meter, respectively. Total anthocyanin was estimated by the pH differential method using two buffer systems: 25 mM K chloride pH 1.0 and 0.4 M sodium acetate pH 4.5[14]. The samples were diluted with K chloride buffer until the absorbance of the sample at 510 nm 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 nm and 700 nm in two different buffers after 15 min of incubation, four times per sample. Total anthocyanin contents were calculated as follows: total anthocyanin = $[(A \times MW \times DF \times 100) / MA]$, where A = (A₅₁₀–A₇₀₀) pH4.5; MW:

molecular weight (449.2); DF: dilution factor; MA: molar absorptive coefficient of cyanidin-3-glucoside (26.900). The results were expressed as mg cyanidin-3-glucoside 100 g^{-1} of juice.

Statistical Analysis

This experiment was carried out with a randomized complete block factorial design with three replications. Data were statistically evaluated by the analysis of variance (ANOVA) to assess the significance of the main factors and the significance of interactions. Combined analysis of variance was carried out assuming environment (years and blocks) as random and treatments as fixed factors. Data were analyzed using SAS software version 9.1, and means were compared using Duncan's multiple range test at $p < 0.05$ level.

RESULTS

Change in Leaf Mineral Concentrations with Foliar Fertilization

The presented results in Table 2 showed that foliar application with 1% and 2% urea alone or in combination with other fertilizers significantly increased leaf nitrogen concentration compared to the control. Nitrogen concentration in the leaves of trees sprayed with 2% urea was significantly higher than 1% urea. The lowest leaf nitrogen concentration (1.52%) was in untreated trees and the highest (3.55%) was urea 2%. The concentration of boron in the leaves of trees sprayed with boric acid 1500 mg/L (24.3%) and 2500 mg/L (24.18%) was significantly higher than the untreated trees (20.04 %). The concentration of iron in the leaves of trees sprayed with iron sulfate 1500 mg/L (125.15mg/kg) and 2500 mg/L (134.21 mg/kg) was significantly higher than the untreated trees (99.17 mg/kg). Also, the concentration of zinc in the leaves of trees sprayed with zinc sulfate 1500 mg/L (24.4 mg/kg) and 2500 mg/L (25.48 mg/kg) was significantly higher than the untreated trees (20.1 mg/kg). Concentrations of urea, boron, iron and zinc on the leaves of trees sprayed with urea 1% + other fertilizers each 1500 mg/L and urea 2% + other fertilizers each 2500 mg/L were significantly higher than the untreated trees. Foliar application of urea, boron, iron, zinc and copper alone or in combination with all fertilizers had no significant effect on the concentrations of phosphorus, potassium, calcium,

magnesium, manganese and copper in the tree leaves.

Yield

Urea 1%, urea 2% and foliar spray of fertilizers with both concentrations (urea 1% + other fertilizers each 1500 mg/L and urea 2% + other fertilizers each 2500 mg/L) increased the yield significantly in comparison with the control. The maximum yield (3.429 kg/tree) and the minimum yield (2.812 kg/tree) were related to the treatments of urea 2% + other fertilizers each of 2500 mg/L and control respectively.

One Fruit Weight

Urea 1% and 2%, foliar spray of fertilizers together (urea 2% + other fertilizers each 2500 mg/L) and iron sulfate 2500 mg/L compared to the control significantly enhanced the fruit weight. Foliar spray of fertilizers together (urea 2% + other fertilizers each 2500 mg/L) rendered maximum fruit weight (0.38 g).

Dry Weight

Urea 1%, urea 2%, boric acid 1500 mg/L, and foliar spray of fertilizers with both concentrations (urea 1% + other fertilizers each 1500 mg/L and urea 2%

+ other fertilizers each of 2500 mg/L) had significantly increased the dry weight in comparison with the control. The maximum dry weight (2.99 g/15 g fresh fruit) and the minimum dry weight (2.20 g/15 g fresh fruit) were related to urea 2% and control respectively.

TSS

Urea 1%, urea 2%, boric acid 1500 mg/L, boric acid 2500 mg/L, and foliar spray of fertilizers with both concentrations (urea 1% + other fertilizers each 1500 mg/L and urea 2% + Other fertilizers each 2500 mg/L) improved the TSS significantly in comparison with the control.

Total Anthocyanin

Urea 1%, urea 2%, boric acid 1500 mg/L, boric acid 2500 mg/L, and foliar spray of fertilizers with both concentrations (urea 1% + other fertilizers each 1500 mg/L and urea 2% + other fertilizers each 2500 mg/L) elevated anthocyanin levels in comparison with the control.

Total Acid

Urea 1%, urea 2%, boric acid 1500 mg/L, and boric acid 2500 mg/L significantly reduced TA in comparison with the control.

Table 2 Effects of Urea, B, Fe, Zn, and Cu on leaf mineral composition. Data shown are means of the two seasons.

Treatment	N (%)	Fe(mg/kg)	Zn(mg/kg)	B(mg/kg)
Urea 0%	1.52 a	98.34 a	18.95 a	19.5 a
Urea 1%	2.21 b	99.10 a	20.32 a	19.5 a
Urea 2%	3.55 c	100.20 a	19.64 a	20.43 a
Boric acid 0 mg/L	1.45 a	100.15 a	19.65 a	20.04 a
Boric acid 1500 mg/L	1.41 a	99.98 a	20.15 a	24.3 b
Boric acid 2500 mg/L	1.53 a	98.74 a	19.45 a	24.18 b
Iron sulfate 0 mg/L	1.43 a	99.17 a	19.3 a	19.8 a
Iron sulfate 1500 mg/L	1.56 a	125.15 b	20.45 a	19.9 a
Iron sulfate 2500 mg/L	1.48 a	134.21 c	20.33 a	20.10 a
Zinc sulfate 0 mg/L	1.50 a	98.95 a	20.1 a	20.13 a
Zinc sulfate 1500 mg/L	1.49 a	99.54 a	24.4 b	19.62 a
Zinc sulfate 2500 mg/L	1.52 a	100.43 a	25.48 b	19.53 a
Copper sulfate 0 mg/L	1.53 a	100.68 a	19.9 a	20.18 a
Copper sulfate 1500 mg/L	1.53 a	99.80 a	20.3 a	20.18 a
Copper sulfate 2500 mg/L	1.49 a	99.59 a	20.13 a	19.70 a
Urea 0% + other fertilizers each 0 mg/L	1.49 a	99.25 a	19.7 a	19.9 a
Urea 1% + other fertilizers each 1500 mg/L	2.26 b	124.95 b	25.1 b	23.9 b
Urea 2% + other fertilizers each 2500 mg/L	3.50 c	134.19 c	24.95 b	24.2 b

Means with the same letter in each column were not significantly different using Duncan's multiple t range test at $p < 0.05$.

Table 3 Effects Urea, B, Fe, Zn, and Cu on the physico-chemical characteristics of Barberry. Data shown are means of the two years.

Fertilizer type and concentration	Fruit weight (g)	Yield (kg/tree)	Dry weight (g/15g FW)	Total Acid (%)	Total anthocyanin (mg/100g FW)	TSS (%)	Juice PH
Urea 0%	0.29 e	2.862 bc	2.22 h	4.35 ac	92.12 c	18.14 c	2.07 bd
Urea 1%	0.34 abcd	3.115 a	2.81abc	3.26 b	127.31 ab	20.17 a	2.65 a
Urea 2%	0.36 ab	3.207a	2.99 a	3.24 b	132.11 a	21.69 a	2.80 a
Boric acid 0 mg/Lit	0.30 de	2.846 bc	2.44 efj	4.36 a	92.15 c	18.16 c	2.09 bd
Boric acid 1500 mg/Lit	0.30 de	2.855 bd	2.90 ab	3.31 b	118.23 b	18.2 b	2.68 ae
Boric acid 2500 mg/Lit	0.33 b	3.045 bc	2.96 ab	3.25 b	291.22 ab	2.60 a	2.66 ae
Iron sulfate 0 mg/Lit	0.30 de	2.877 bc	2.23 jh	4.32 a	92.21 c	18.55 b	2.07 bd
Iron sulfate 1500 mg/Lit	0.30 de	2.979 bc	2.76 abcde	4.03 ac	94.31 c	20.56 a	2.33 ac
Iron sulfate 2500 mg/Lit	0.36 abc	3.184 ab	2.80 abcd	3.95 ac	91.17 c	21.16 a	2.42 ceb
Zinc sulfate 0 mg/Lit	0.30 de	2.829 c	2.36 efjh	4.35 ac	92.41 c	18.13 c	1.85 d
Zinc sulfate 1500 mg/Lit	0.30 de	2.933 bc	2.34 efjh	4.36 a	92.35 c	18.21 c	1.97 d
Zinc sulfate 2500 mg/Lit	0.29 de	2.838 bc	2.22 jh	4.32 ac	90.23 c	18.34 c	2.08 bc
Copper sulfate 0 mg/Lit	0.29 de	2.859 bc	2.24 fjh	4.35 ac	92.22c	18.16 c	2.08 bc
Copper sulfate 1500 mg/Lit	0.29 de	2.844 bc	2.32 fjh	4.31 ac	92.36 c	18.33 c	2.03 d
copper sulfate 2500 mg/Lit	0.28 de	2.831bc	2.20 h	4.35 ac	94.32 c	18.33 c	2.01 d
Urea 0% + other fertilizers each 0 mg/Lit	0.29 e	2.812 c	2.20 h	4.36 a	92.24 c	18.14 c	2.08 bc
Urea 1% + other fertilizers each 1500 mg/Lit	0.33 bcd	3.172 a	2.71 abcde	3.44 b	130.24 ab	20.74 a	2.65 ae
Urea 2% + other fertilizers each 2500 mg/Lit	0.38 ab	3.429 a	2.80 abc	3.24 b	129.22 ab	21.66 a	2.80 a

Juice pH

Urea1%, urea 2%, boric acid 1500 mg/L, Boric acid 2500 mg/L, and foliar spray of fertilizers with both concentrations (urea1% + other fertilizers each 1500 mg/L and urea 2% + Other fertilizers each 2500 mg/L) increased pH as compared with the control (Table3).

Other fertilizers: Boric acid + Iron sulfate + Zinc sulfate + Copper sulfate. Means with the same letter in each column were not significantly different using Duncan's multiple t range test at $P < 0.05$.

DISCUSSION

Despite its long history of cultivation, fertilizer requirements for barberry are not understood because of the absence of research. Barberry is grown only in the South Khorasan province located in the east of Iran.

With more intensive barberry farming, diagnostic of leaf nutrient concentration standards need to be established and the effects of cultural methods such as foliar nutrient applications on the barberry yield and physico-chemical properties of fruits need to be explored in detail. Foliar nutrient sprays are utilized extensively in agricultural crop production, not only to correct nutrient deficiencies and improve sustainability by way of targeted fertilizer

applications but also to improve plant productivity by providing nutrients at key phenological stages or when nutrient uptake or mobility is limited [8]. However, the effectiveness of foliar nutrient applications is dependent on several factors, including but not limited to species and/or cultivar, stage of phenological development, and nutrient uptake and translocation [15,16]. This indicates that foliar nutrient uptake occurred, suggesting that these nutrients could be applied foliar to affect plant nutrient status. Hasani *et al.* [17] also found that leaf Zn concentrations were significantly increased by $ZnSO_4$ applications to 'Malas e Torsh e Saveh' pomegranate. Davarpanah *et al.* [18] reported significant increases in leaf Zn concentrations of 'Ardestani' pomegranate in response to foliar nano-Zn chelate fertilizer but only at the highest Zn concentration tested or when applied in combination with foliar B. It suggests that fertilizer formulation and concentration and/or cultivar could influence the efficacy of foliar Zn applications for increasing leaf Zn concentrations of pomegranate. However, in this study, foliar application of all fertilizers did not have a significant effect on leaf nutrient content compared to foliar application of fertilizers alone. For many fruit tree crops, studies have been conducted to determine correlations between leaf

nutrient concentration and yield but such information is not available for barberry. Therefore, in some studies of foliar nutrient applications to barberry, treatments might have corrected an undetected nutrient deficiency while in others, nutrient concentrations might have already been sufficient to maximize yield. Barberry specific foliar nutrient reference standards are therefore needed to enable growers to sustainably maximize yield and to determine if foliar nutrient applications can be used to correct nutrient deficiencies.

In this test, urea 1% and urea 2% alone as well as combined with other fertilizers increased fruit weight and barberry yield significantly. Concomitant with the results of the current study, reported spraying with urea 2%, urea 3% and urea 2% + zinc 0.8% has increased the jujube yield in comparison with the control significantly [19-20, 9]. Spraying with urea has increased other fruit yields like Asgari grapes and almonds [7,10]. Researchers have indicated that urea has a positive effect on the yield due to the effect of urea on reducing flower and fruit loss [8]. Urea has been considered a rich source of nitrogen for leaves and fruits. Nitrogen has been involved in the production of enzymes. Enzymes boost the formation of meristem cells, cell division, and cell size [4], increasing the amount of nitrogen, the number of enzymes and the fruit and yield. Also, urea has effects on the activity of enzymes that alter photosynthesis and the amount of carbohydrates, and subsequently the dry matter and product yield of plants [6,9]. Nitrogen spraying in the spring leads to stimulation of amino acid formation which increases vegetative growth, leaf area and photosynthesis. The store of carbohydrates will increase, and as a result, elevating fruit formation and yield [7].

In this study, $ZnSO_4$ did not increase barberry yield. According to the findings of this experiment, Hasani *et al.* [5] and El-Rhman [21] reported that foliar applications of $ZnSO_4$ did not affect the yield of 'Malas e Torsh e Saveh' and 'Manfaluty' pomegranate respectively. Khorsandi *et al.* [22] also reported that foliar $ZnSO_4$ did not improve the overall yield but did decrease the unmarketable yield.

The results of the current research indicated that boric acid did not have any significant effect on the fruit weight and yield of barberry. Contrary to the results of this experiment reported that boron has a

significant effect on the fruiting process. Boron deficiency reduces the number of flowers that make up the fruit. Boron spraying prior to full bloom has enhanced fruit formation in Italian plums, pears, cherries, almonds, apples, oranges, and scallops. Boron deficiency will reduce the transfer of sugar to flowers for their growth and the amount of nectar. This issue indicates that boron increases pollination activity during flowering. Increasing the cell division and synthesis of nucleic acid, during fruit development, affects fruit formation [23]. Also, the boron can decrease loss and increase fruit formation, fruit size and yield due to the transport of carbohydrates via a sugar-boron complex that crosses easier than sugar from the membrane. Also, it has such an effect because of the inhibition of the activity of the IAA oxidase enzyme that sustains auxin in the plant and increases the production of cytokinin [23,24].

In this experiment, urea, boric acid and all fertilizer together with all foliar concentrations significantly reduced TA. In line with the results of current research, Bhati and Yadav [9] reported that spraying urea reduces the jujube acidity. Contrary to the results of this experiment, urea spraying increased the TA of the tamarind and pomegranate [19,4].

In the current research, copper sulfate did not have any effect on TA. But copper spraying on grapefruit increased the number of acids in comparison with the control [19].

In the current experiment, the foliar application of urea, boric acid and all fertilizer together significantly increased barberry fruit TSS as found in other studies [9].

In this experiment, urea, boric acid and all fertilizer together with all foliar concentrations significantly increased the amount of anthocyanin fruits. Spraying of boron in peach, as well as the results of this experiment, will improve the color of the fruit but does not affect the colors of apples. According to the findings of this study, boric acid increased the amount of anthocyanin of bell pepper [4].

CONCLUSION

Overall, this study demonstrated that foliar applications of urea, boric acid, iron sulfate and zinc sulfate resulted in significant changes in leaf nutrient concentrations. Based on the results obtained from this experiment, foliar application of urea is recommended to increase the yield and

improve the properties of barberry fruits. Foliar application of boric acid will also improve barberry fruit properties.

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