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Original article

# The influence of foliar application of urea, zinc chelate and boric acid on growth characteristics, photosynthesis and survival of date palm offshoots (*Phoenix dactylifera* cv. Mazafati)

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## Summary

**Date palms are a much neglected plant group in terms of mineral nutrition, especially foliar fertilization. In the present study, the effect of foliar application of urea (46% nitrogen), zinc chelate (40% zinc) and boric acid (17% boron) were evaluated on growth characteristics, photosynthesis and leaf nutrients of 'Mazafati' date palm offshoots. In addition, the survival percentage of date palm offshoots was recorded after offshoot transplanting to the main field. The experiment was carried out as completely randomized design in a controlled greenhouse with seven treatments and 10 replicates. The treatments were control, urea (0.5 and 1%), zinc chelate (1000 and 2000 ppm) and boric acid (2000 and 4000 ppm). Results illustrated that growth parameters of date palm offshoots were improved by foliar application of the nutrients. The maximum leaf and root growth were recorded for urea 1% and zinc chelate 1000 ppm. Chlorophyll *a*, *b* and total, leaf relative water content, protein and reducing sugars were increased by foliar application of the nutrients. Moreover, foliar fertilization of N, Zn and B improved leaf net photosynthesis rate and stomatal conductance. The results of this research illustrated the high percentage of offshoot mortality (~50%) via direct transfer of offshoots to the main field. It has been highlighted that indirect transfer by preserving the offshoots in greenhouse condition and foliar application of both urea and zinc chelate provide growers with a tool to increase offshoots survival (>85%) and revenue.**

**Keywords**
mineral elements, offshoot maintenance, protein, reduced sugars, stomatal conductance

## Significance of this study

*What is already known on this subject?*

- Foliar application of nitrogen, zinc and boron on mature fruit trees have improved growth characteristics, fruit quality and the tree yield. To our knowledge, although the survival percentage of direct transferred date offshoots in the main field is low, foliar fertilization of date offshoot has not been studied yet.

*What are the new findings?*

- Foliar fertilization of nitrogen and zinc can significantly improve leaf and root growth as well as increasing leaf photosynthesis rate and stomatal conductance. Preserving the date offshoots in the greenhouse condition and proper foliar fertilization of offshoots improved offshoot survival percentage (>85%) in the main field.

*What is the expected impact on horticulture?*

- Our results provide practical information on date offshoot preserving in greenhouse condition. In addition, date offshoots exhibited a positive response to foliar fertilization prior to transplanting to the main field. Optimizing date offshoot fertilization program provides growers with a tool to increase offshoots survival and revenue.

## Introduction

Date palm (*Phoenix dactylifera* L.) is the most common fruit tree grown in arid and semi-arid regions of the Middle East, North Africa and South USA (Chao and Krueger, 2007). Iran, with 198,000 ha bearing area harvest and 162,000 ton production, is among the major date producing countries (IMAJ, 2015). 'Mazafati' is the most important commercial date cultivar grown in Kerman province, Iran, which is propagated asexually from separation of true to type offshoots. Today, foliar fertilization has become a common practice to overcome macro and micro nutrients deficiencies which efficiently meets the nutrient demand of plants during periods when macro and micro elements become fixed in the soil and soil-applied fertilizers render ineffective (Silberbush, 2002; Lovatt, 2013). Nitrogen (N), zinc (Zn) and boron (B) are among the mineral nutrients which play important roles in almost all plant metabolic processes and expedites normal plant growth and development (Pilbeam, 2015; Barker and Pilbeam, 2015; Welch, 1995). In the previous studies regarding foliar application of nitrogen, zinc and boron on mature fruit trees, significant improved growth and boosted fruit quality and yield were reported (Sayyad-Amin and Shahsavar, 2012; Nikkhah et al., 2013; Gurel and Basar, 2016). In addition, it has been reported that foliar and soil applications of mineral nutrients improve the growth and survival of some forest trees saplings (Ceccon et al., 2003; Oskarsson et al., 2006; Chaplot, 2013).

Although economically important in arid and semi-arid parts of the world, date palms are a much neglected plant group in terms of mineral nutrition especially foliar appli-

cation of mineral nutrients. Although a few studies regarding foliar fertilization of mature date palm trees have been carried out (Khayyat et al., 2007; Sarrwy et al., 2012), to our knowledge, foliar fertilization of date palm offshoots has not been investigated yet. Moreover, our information about survival percentage and maintenance of date offshoots in the main field is limited. Although direct transfer of separated offshoots from parent plants to the main field is a common horticultural practice in Middle East countries, especially Iran, to our knowledge, survival of such offshoots in the main field is low. Thus, the objectives of this study are (1) to understand if foliar fertilization of nitrogen, zinc and boron can be effective in efficient utilization of these nutrients by date offshoots and thereby improve growth characteristics and photosynthesis rate, and (2) to find out if indirect transfer of offshoots (preserving the offshoots in greenhouse condition) and foliar fertilization by such mineral elements increase the survival and maintenance of date offshoots in the main field.

## Materials and methods

### Date palm offshoot preparation and treatments

The experiment was carried out in a controlled greenhouse at Shahid Bahonar University of Kerman (SBUK), Iran. The research was carried out on 'Mazafati' date palm offshoots grown in 40-L plastic plant pots containing 80% soil and 20% peat. In order to optimize the soil nutrients status, additional nitrogen ($NH_4NO_3$) and phosphorus ($KH_2PO_4$) were applied uniformly to all pots. The characteristics of the soil are shown in Table 1. The selected offshoots were approximately similar in age (four-year-old), weight (9 kg), base diameter (20 cm) and number of leaves (3 leaves) with the signs of maturity as the availability of their own roots. In order to prevent water stress, the irrigation schedule was set as 5-day intervals for the first three weeks and 10-day intervals until the end of experiment. After three months of adaptation in greenhouse condition, the offshoots were arranged as completely randomized design with seven foliar treatments and 10 replicates as follows:

- Control (distilled water);
- Urea (0.5% and 1%), (46% pure nitrogen, Merck Co., Germany);
- Zinc chelate (1000, 2000 ppm), (40% pure zinc, Zintrac®, Yara Co., Germany);
- Boric acid (2000, 4000 ppm), (17.4% pure boron, Merck Co., Germany).

Treatments were applied twice with 10-day intervals and 100 mg $L^{-1}$ Tween® 20 (Sigma-Aldrich Co. LLC) was used as a surfactant. In order to avoid dripping down onto the soil during spray applications, the soil was temporarily covered with a greenhouse clear plastic film polyethylene.

### Morphological, physiological and biochemical analyses

All morphological, physiological and biochemical analyses were carried out 10 weeks after the second foliar application. Leaf area, leaf fresh and dry weights, root volume and root fresh and dry weights were measured as followed. In order to measure leaf dry weight, the samples were oven-dried at 75°C for 48 h until they reached a constant weight. Thereafter, they were weighed and finely ground. Leaf area was determined by a digital leaf area meter (ADC BioScientific Ltd., UK). At the end of experiment, four offshoots from each treatment were cut at soil surface and the roots washed free of soil. Root dry weights were measured as the procedure described for leaf samples. The root volume was recorded using an actual volume displacement technique involving submerging roots in a graduated cylinder to observe the volume of water displaced (Burdette, 1979).

The amount of photosynthetic pigments (chlorophyll *a*, *b*, total chlorophyll and carotenoids) were determined according to the method of Lichtenthaler (1987).

Leaf relative water content was determined by the method described by Wheutherley (1950) and estimated as following formula:

$$RWC\ (\%) = [(\mathrm{FW} - \mathrm{DW})(\mathrm{TW} - \mathrm{DW})] \times 100$$

where FW = Fresh weight (g); DW = Dry weight (g); TW = Turgid weight (g).

For reducing sugars analysis, fresh leaf samples (0.02 g) were ground in 15 ml of deionized water and then boiled for 0.5 min. After extraction, concentration of reducing sugars was measured by the method described by Somogyi (1952). The absorbance was measured at 600 nm by spectrometer (UV-VS, Cary, USA) and glucose was used as standard solution.

For analyzing protein, fresh leaf samples (1 g) were ground in 5 ml Tris-HCl buffer (0.05 M). The homogenates were centrifuged at 10,000 *g* for 25 min at 4°C. Then, the protein content in the supernatant was analyzed according to the method of Bradford (1976).

### Gas-exchange measurements

Gas exchange measurements were performed using LCA-4 portable photosynthesis system (ADC BioScientific Ltd., Hoddesdon, UK). The air-flow rate was maintained at a constant rate of 500 µmol $s^{-1}$ and the reference $CO_2$ concentration was about 400 µmol $mol^{-1}_{air}$. All measurements were carried out between 09:00 and 11:00 am. During the measurement the relative humidity was about 60% and the leaf temperature ranged from 23-25°C.

### Mineral elements analyses

Mineral nutrient analyses were carried out for the leaf samples. Fresh leaflets (1 g) were washed with deionized water, ground and ashed at 550°C for 8 h. Nitrogen was determined by the Kjeldahl method (AOAC, 1985) and expressed as percentage. Leaf zinc content was measured using an absorption spectrophotometer (Model contrAA 700 , Analytikjena Co., USA). Leaf boron concentration was analyzed according to Azomethine-H method described by Wolf (1974). The concentrations of Zn and B were expressed as mg $kg^{-1}$ on a dry weight basis.

### Offshoot survival determination in the main field

At the end of experiment, the offshoots were transferred to the main field located in Bam region, Kerman province, Iran. Climatic conditions of Bam region and soil character-

**Table 1.** Some chemical and physical properties of soil used in the experiment.

| Texture | Sand | Silt | Clay | Total N | Absorbable K | Absorbable P | SAR | pH | EC |
|---|---|---|---|---|---|---|---|---|---|
| Loam | 42% | 38% | 21% | 0.18% | 245 mg $kg^{-1}$ | 16 mg $kg^{-1}$ | 5.2 | 7.8 | 1.2 dS $m^{-1}$ |

SAR: sodium adsorption ratio.

**Table 2.** Climatic conditions of Bam region in Kerman province, Iran.

| Location | Climate | Max. temp | Min. temp. | RH | Rainfall | Wind speed |
|---|---|---|---|---|---|---|
| Bam, Kerman province, Iran (lat. 29°6'N; long. 58°20'E) | Warm and dry desert climate | 47.6°C | -8.6°C | 30% | 53 mm | 5 m $s^{-1}$ |

**Table 3.** Some soil chemical and physical properties of main field in Bam region.

| Depth | Texture | Sand | Silt | Clay | Total N | Absorbable K | Absorbable P | SAR | pH | EC |
|---|---|---|---|---|---|---|---|---|---|---|
| 0-40 cm | Sandy loam | 60% | 26% | 14% | 0.09% | 210 mg $kg^{-1}$ | 13 mg $kg^{-1}$ | 5.7 | 7.9 | 1.7 dS $m^{-1}$ |
| 40-80 cm | Loam | 51% | 37% | 12% | 0.07% | 195 mg $kg^{-1}$ | 11 mg $kg^{-1}$ | 6.3 | 8.2 | 2.4 dS $m^{-1}$ |

istics of the main field are shown in Tables 1 and 2. In order to determine the offshoots survival in the main field and to figure out the difference between direct and indirect transfer of separated offshoots, six true to type mature 'Mazafati' offshoots similar in size and weight to the greenhouse offshoots were separated and directly transferred and cultured with the greenhouse offshoots based on a randomized complete block design (RCBD) with six replications (blocks). The offshoots were subjected to the common horticultural practices and 7-day irrigation intervals. Four months after transplanting, the percentage of offshoot survival in the main field was determined.

**Statistical analysis**

Analyses of variance were performed using the General Linear Models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC, USA). The means were separated using Duncan's multiple range test at $p < 0.05$.

## Results and discussion

The results showed that the foliar application of urea, zinc chelate and boric acid improved the leaf and root growth parameters. Urea (1%) and zinc chelate (1000 ppm) were the most effective treatments in improving the offshoot leaf parameters including leaf area as well as leaf fresh and dry weights (Table 4). Moreover, root volume and root fresh and dry weights were significantly boosted by foliar fertilization of the elements rather than control plants (Table 4) which is in accordance with the previous studies (Hafeez et al., 2013; Chilundo et al., 2017).

Leaf chlorophyll *a*, *b* and total were significantly affected by foliar application of urea, zinc chelate and boric acid. The least chlorophyll (*a*, *b* and total) contents were related to control offshoots while the foliar fertilized offshoots showed significant higher chlorophyll contents (Figure 1) which might be ascribed to the involvement of N, Zn and B as structural and catalytic components and as co-factor for normal biosynthesis of chlorophyll and pigments (Balashouri, 1995; Fageria, 2009; Mitra, 2015).

According to the results, foliar application of urea, zinc chelate and boric acid significantly increased RWC of treated offshoots compared to the control. The highest RWC (79%) was recorded for the foliar treated offshoots by zinc chelate 1000 ppm while the least RWC (66%) was observed for control plants. However, there were no significant differences between urea and zinc chelate fertilizers (Figure 2).

This study showed that foliar application of N, Zn and B can affect leaf protein and reduced sugars (Figure 2). The highest leaf protein content was related to urea 1% and zinc chelate 1000 ppm (93.3 and 90.6 mg, respectively), while the least protein content (72.4 mg) was observed for control offshoots (Figure 2). It has been found that nitrogen as a major component of amino acids, the building blocks of proteins, and zinc as a cofactor for RNA polymerase enzyme have fundamental roles in biosynthesis of amino acids and proteins as well as stability and function of genetic materials and ribosomes (Cakmak et al., 1989; Alloway, 2008; Mitra, 2015). Moreover, the highest amount of reduced sugars (56 mg) was recorded for the offshoots fertilized by boric acid (4000 ppm) while the least content (38 mg) was related to the control plants (Figure 2) which is in accordance with the previous study by Saadati et al. (2013) on olive trees. It seems that boron plays important roles in photosynthesis, sugar transport as well as carbohydrate and amino acid metabolism (Lu et al., 2014).

According to our study, foliar application of N, Zn and B significantly increased the leaf net photosynthesis rate and stomatal conductance (Figure 3) which can be ascribed to significant increase of leaf chlorophyll contents and RWC of foliar fertilized offshoots than control plants (Figures 1 and 2). The increased leaf chlorophyll content and photosynthesis rate by N application indicate that more N may be allo-

**Table 4.** Effect of foliar fertilization of urea, zinc chelate and boric acid on leaf and root parameters of 'Mazafati' date palm offshoots.

| Treatment | Leaf area ($m^2$) | Leaf FW (g) | Leaf DW (g) | Root volume ($cm^2$) | Root FW (g) | Root DW (g) |
|---|---|---|---|---|---|---|
| Control | 0.22 ± 0.02 c* | 107 ± 8.45 c | 50.4 ± 8.5 c | 265 ± 18 c | 230 ± 20 c | 74.0 ± 4.0 c |
| Urea 0.5% | 0.26 ± 0.03 bc | 149 ± 10.5 b | 62.3 ± 6.4 bc | 335 ± 15 b | 290 ± 12 b | 91.1 ± 5.2 ab |
| Urea 1% | 0.31 ± 0.01 a | 168 ± 13.5 ab | 69.3 ± 3.2 b | 380 ± 12 a | 325 ± 8.2 a | 102.1 ± 6.7 a |
| Zn Chelate 1000 ppm | 0.33 ± 0.02 a | 189 ± 12.5 a | 83.2 ± 7.65 a | 330 ± 17 b | 280 ± 11.3 b | 87.9 ± 5.4 ab |
| Zn Chelate 2000 ppm | 0.24 ± 0.02 c | 113 ± 9.33 c | 60.1 ± 3.4 c | 318 ± 15.3 b | 270 ± 8 b | 87.0 ± 3.5 bc |
| Boric acid 2000 ppm | 0.25 ± 0.021 c | 123 ± 7.5 c | 61.6 ± 3.89 c | 275 ± 15.9 c | 240 ± 10 c | 75.36 ± 3.4 c |
| Boric acid 4000 ppm | 0.24 ± 0.01 c | 114 ± 8.9 c | 58.3 ± 5.2 c | 282 ± 14 c | 260 ± 10 bc | 81.0 ± 5.2 bc |

* Values are mean ± SE, where $n = 10$; each column followed by the same letter is not significantly different according to Duncan's multiple range test ($P < 0.05$).

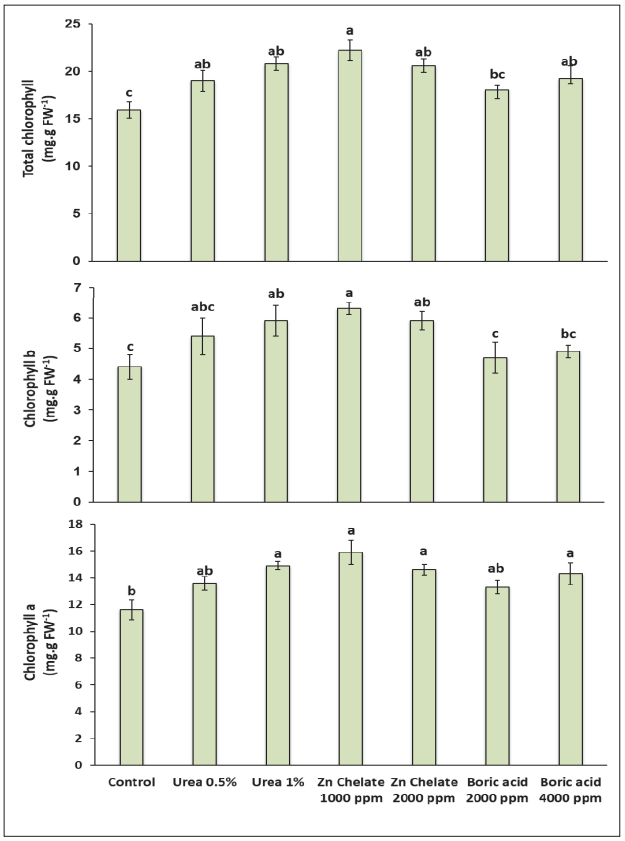



**Figure 1.** Effect of foliar fertilization of urea, zinc chelate and boric acid on leaf chlorophyll contents of 'Mazafati' date palm offshoots in greenhouse condition. Each column represents an average ± SE, $n = 10$.

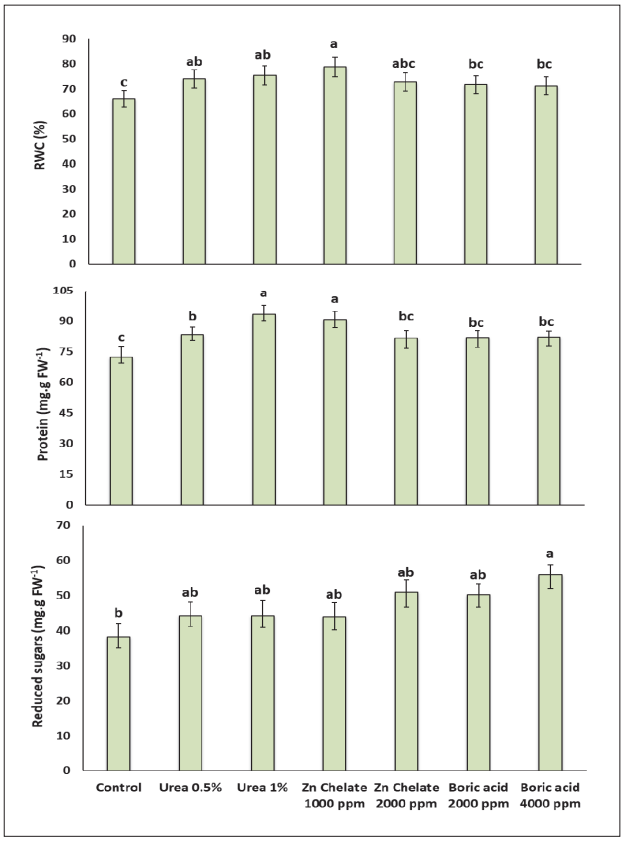



**Figure 2.** Effect of foliar fertilization of urea, zinc chelate and boric acid on leaf relative water content, protein and reduced sugars of 'Mazafati' date palm offshoots in greenhouse condition. Each column represents an average ± SE, $n = 10$.

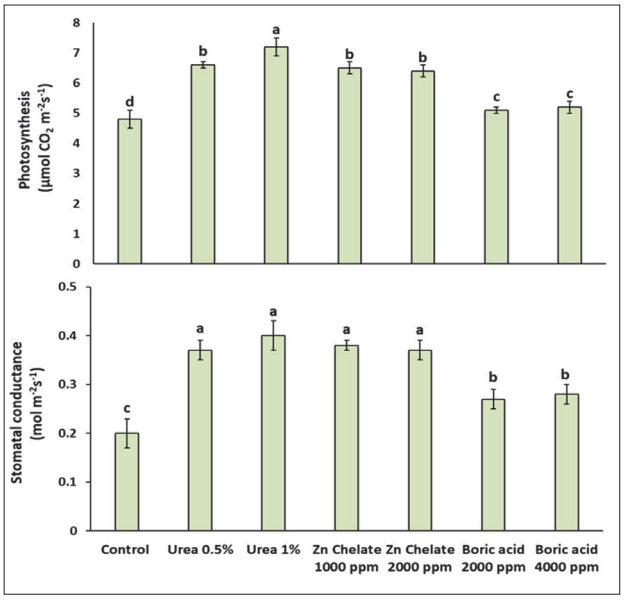



**Figure 3.** Effect of foliar fertilization of urea, zinc chelate and boric acid on leaf net photosynthesis and stomatal conductance of 'Mazafati' date palm offshoots in greenhouse condition. Each column represents an average ± SE, $n = 10$.

cated to the enzymes of the carbon reactions such as Rubisco and more $CO_2$ could be used by the plant (Foyer et al., 2001). Many plant biological processes such as $CO_2$ fixation, maintenance of biological membranes, auxin synthesis and regulatory protein synthesis are controlled by zinc containing enzymes. Zinc also plays a role as a cofactor of some enzymes including SOD, CAT and alcohol dehydrogenase which act as ROS scavenging and improving photosynthesis (Mathpal et al., 2015).

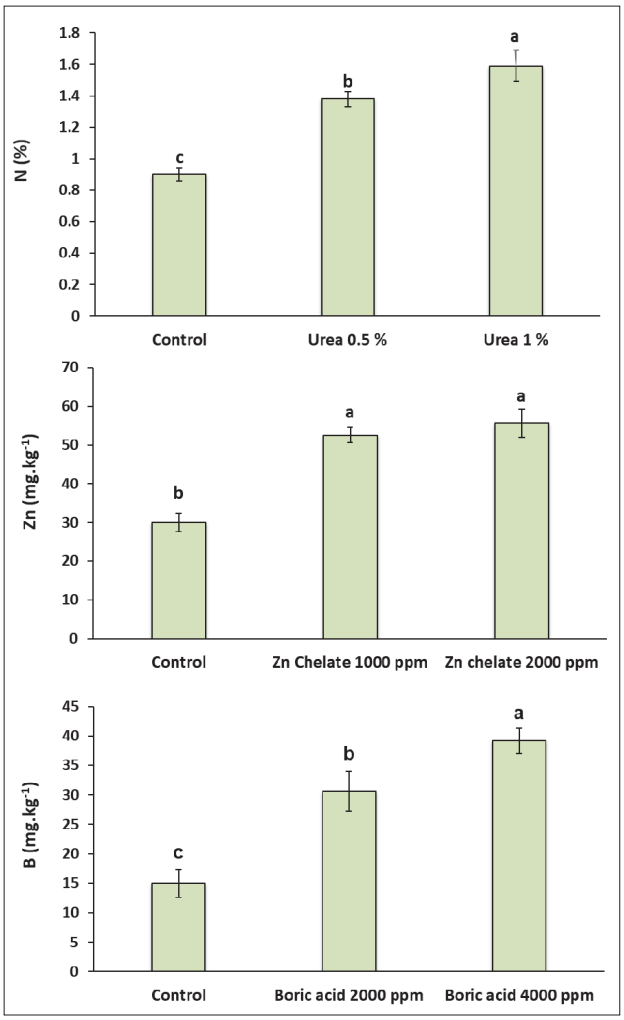



**Figure 4.** Effect of foliar fertilization of urea, zinc chelate and boric acid on leaf nitrogen, zinc and boron contents of 'Mazafati' date palm offshoots in greenhouse condition. Each column represents an average ± SE, $n = 10$.

Our results showed that foliar fertilization of urea, zinc chelate and boric acid significantly increased leaf N, Zn and B concentrations respectively (Figure 4) representing the positive response of date offshoot leaves to absorb the nutrients via foliar application, although the offshoot leaf has a thick waxy cuticle (Zaid and Arias-Jiménez, 2002).

According to the results, there was a significant difference between direct transferred offshoots (52%) and greenhouse offshoots (75%) in terms of offshoot survival in the main field. The highest survival was recorded for both urea and zinc chelate treated offshoots (>85%), although no significant difference was found among N, Zn and B fertilizers (Figure 5). This indicates that direct transferred offshoots to the main field, which is a common horticultural practice in Iran and some Middle East countries, leads to low survival percentage and thus should be avoided. In other words, preserved offshoots in the greenhouse or even in a nursery for 3–5 months can significantly survive more in the main field, mainly due to better root regeneration. The limitation of date offshoot maintenance in the main field might be affected by various factors including the inappropriate type of offshoot, inadequate and poor roots, inattention during offshoot separation and transferring to the soil, biotic and abiotic stresses and poor nutrient contents of offshoots. Our results showed that preserving the offshoots in the greenhouse and proper foliar fertilization of offshoots especially with appropriate N and Zn fertilizers can significantly cause more leaf and root growth, improved photosynthesis and subsequently more offshoot survival in the main field.

## Conclusion

To our knowledge, this is the first report on foliar fertilization of date offshoot and its survival in the main field. Taken together, the results of this research showed that with the high percentage of date palm offshoot mortality (~50%) in the main field, indirect transfer of offshoots as well as foliar fertilizers provide growers with a tool to increase offshoots survival and revenue. According to the results of the present study, foliar fertilization of urea 1%, zinc chelate 1000 ppm and boric acid 4000 ppm improved the vegetative growth and photosynthesis rate and successfully maintained 'Mazafati' date palm offshoots in the main field. However, this is a preliminary study and much work remains to elucidate the effect of other mineral elements as well as the combination of macro and micro mineral nutrients to improve the growth characteristics, their impacts on photosynthesis rate as well as their effects on date offshoot survival in the main field.

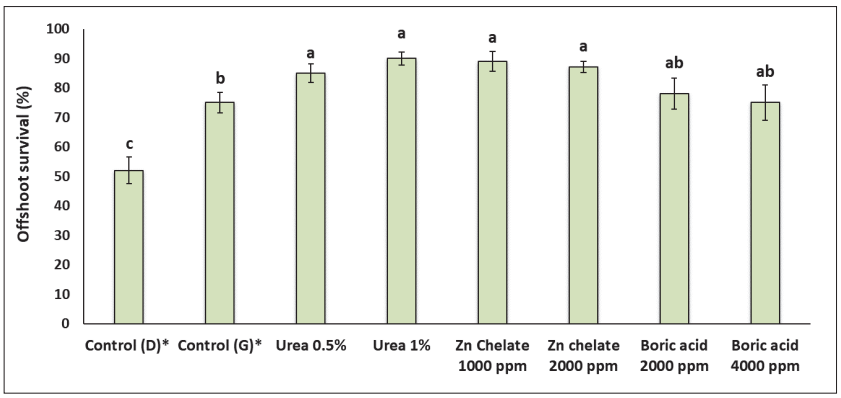



**Figure 5.** Effect of foliar fertilization of urea, zinc chelate and boric acid on survival of 'Mazafati' date palm offshoots in the main field. Control (D)*= offshoots transferred directly to the main field after separation from parent palm. Control (G)**= offshoots transferred indirectly to the main field after separation from parent palm and preserving in greenhouse condition. Each column represents an average ± SE, $n = 6$.

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