



Study of Biochemical Traits and Mineral Elements in Date Palm Fruits under Preharvest Foliar Application of Organic Fertilizers and Micronutrients

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ABSTRACT

Due to the calcareous nature of most soils in areas under date cultivation, this study was conducted to improve the quality and quantity of date fruits in Zahidi cultivar using the foliar applications of organic matters and micronutrients at two stages of date growth. For this purpose, an experiment was conducted to evaluate the effects of foliar applications of organic matters and micronutrients on contents and properties of biochemical compounds and minerals in Zahidi date fruit. The study was performed as a randomized complete block design with 11 treatments in three replications on 33 date palms for two consecutive years. The treatments were applied in two stages (at the beginning and end of the Kimri stage) on fruits and upper leaves. Treatments included amino acids, Aminabon 50 (0.5 and 0.1 g L⁻¹), seaweed (0.25 and 0.5 g L⁻¹), micronutrients (0.1 and 1.5 g L⁻¹), and four combined treatments obtained from different concentrations of amino acids, seaweed, and micronutrients. Spraying with distilled water served as the control treatment. The results showed that the treatments had a significant effect on all studied traits. The highest content of copper was observed in response to amino acid + micronutrients (1.63 mg kg⁻¹). The effects of amino acid + seaweed + micronutrients on the other traits were observed in the highest statistical class. In general, the latter combined treatment was the most efficient with the lowest content of soluble tannin (26.46 mg g⁻¹) compared to the control (32.12 mg g⁻¹) and to the other treatments.

Introduction

Date palm (*Phoenix dactylifera*; *Araceae*) is a monocotyledonous plant, i.e. male and female flowers are on separate trees. In terms of fruit production, the economic life of date palms is about 40 years (Mostaan et al., 2017). Zahidi cultivar is one of the dry and marketable cultivars and is one of the rarest cultivars whose fruit color is light yellow in the Tamar stage. In addition to domestic consumption, its fruit has good

marketability for export. Due to the dryness of the tissue, the fruit can be easily transported. Due to its unique characteristics such as adaptability to different climatic conditions, cold tolerance, and relative resistance to date-spider mite [*Oligonychus afrasiaticus* (McG.)], Zahidi cultivar is cultivated in most date-growing regions (Mostaan et al., 2017). The main growing areas of Zahidi date palm have mainly calcareous soils, with limited availability of nutrients. Therefore, to

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solve this problem and increase absorbable nutrients, it is necessary to take special measures in these types of soils. One of these solutions is foliar application such as foliar application or other integrated management practices (Aghaye Norrozlo et al., 2019; Aslani and Sour, 2018). The foliar application of minerals and organic matters primarily improves the nutritional status of the aerial parts of the trees. These treatments activate molecular, physiological, and biochemical processes in trees. The signals of these changes are transmitted from the leaves to the aerial parts as well as the underground part of the plant. Mineral elements also play an activating or cofactor role in regulation or stimulation of enzymatic processes in fruits (Srivastava, 2012). Using more inputs in orchard management, especially chemical fertilizers, is considered as one of the main components that can increase yield, but this has led to serious environmental problems in recent decades. Today, one of the basic solutions to overcome these problems is the use of biological and organic fertilizers. Commercial bio-stimulants include important compounds such as seaweed extracts, plant hormones, humic substances, amino acids, etc. The compounds are mainly considered in agriculture, animal husbandry, and fishery sections (Araujo et al., 2011). From the point of view of farmers, one of the main components of increasing the yield of agricultural products is the use of more inputs, especially chemical fertilizers, while their optimal and timely use is more effective. Unfortunately, the increasing of using of chemical fertilizers in recent decades has led to enormous environmental problems.

The use of organic fertilizers can help to solve the problems, including gradual degradation of soil quality, reduction of product quality. It prevents disturbing the natural balance of the ecosystem due to spread of environmental pollution. Plant growth stimulants (amino acids) can stimulate plant growth and development under optimal and stress conditions (Ronga et al., 2019). These substances can also regulate and enhance the physiological processes of plants. Growth stimulants act in plant physiology in many ways, including improving product growth, yield, quality, nutrient uptake, and tolerance to abiotic stress factors (Yakhin et al., 2017). The roles of amino acids include regulation of ion transport, stomatal closure, reduction of heavy metal toxicities, and acting as osmolytes (Anjum et al., 2014). Amino acids affect the growth and yield of plants by increasing their tolerance to environmental stresses, increasing chlorophyll contents and consequently photosynthesis (Pouryousef-Miandoab and Shahravan, 2014).

Seaweed extract is one of the growth stimulants compounds and unlike chemical fertilizers, it prevents environmental degradation. It is non-toxic, and does not cause dangerous pollution for humans, animals, and birds. Seaweeds are commonly found in shallow seawater and groundwater, and their extracts contain hormones such as auxins, gibberellins, and cytokinins, and recently small amounts of brassinosteroids, jasmonate, and salicylic acid have been reported (Erulan et al., 2009). In addition, seaweeds contain micronutrients such as iron, cobalt, magnesium, molybdenum, zinc, and nickel, and also contain vitamins and amino acids (Erulan et al., 2009). Previous research has shown that seaweed extract stimulates plant growth and yield, and increases their resistance to a variety of environmental stresses (Pramanick et al., 2013). Algae contain hormones such as auxins, gibberellins, cytokines, brassinosteroids, jasmonates, and salicylic acid. They also contain micronutrients such as iron, cobalt, magnesium, molybdenum, zinc, and nickel. Algae also contain vitamins and amino acids (Erulan et al., 2009). In addition, they contain amino acids, and these substances have different hormones or can cause these processes by affecting enzymes.

Abiotic factors such as nutritional deficiencies cause the disorders in the syntheses of carbohydrates, protein, and organic matters in plants, thereby reducing the rate of growth and development in trees and, ultimately, reducing crop production, which severely weakens the trees. Optimal nutrition of fruit trees is one of the effective ways to achieve maximum fruit yield, increase the quality of the product, and also increase their storage capacity (Brunetto et al., 2015). In the growing date palm areas, due to high pH, abundant lime in the soil, and lack of organic matter in soils, uptakes of iron, zinc, and other micronutrients by plants are usually low and under such conditions, micronutrient deficiencies are observed (Mirzapour and Khoshgoftarmanesh, 2013). Iron plays a direct role in photosynthesis, respiration, and nitrogen fixation, as well as roles in the activity of enzymes and electron transfer, and acts as an enzyme activator or cofactor in chlorophyll production (Mahdavia and Mahna, 2012). Zinc is a micronutrient that is needed for the formation and production of the appropriate size of the fruit. The element is present in part of the carbonic anhydrase enzymes in all photosynthetic tissues, which is required for chlorophyll biosynthesis. Zinc is also involved in the production of tryptophan, a precursor in the synthesis of auxin (Azadi and Gharaghani, 2016). Foliar application of the element had positive effects on the

formation, improvement, and yield of the fruit, due to its positive role in the percentage of germination pollen grain (Ahmed et al., 2002). Due to the calcareous nature of most soils in the date cultivation areas, this study was conducted to improve the quality and quantity of date fruits in Zahidi cultivar using the foliar applications of organic matters and micronutrients in two stages of date growth periods.

Materials and Methods

This experiment was carried out in two crop years, 2018-2019 and 2019-2020, as a randomized complete block design with 11 treatments and in three replications on 33 same-age date palms of Zahidi cultivar in Jahrom, Iran. The treatments were performed in the early Kimri stage (first half of June) and then in the late Kimri stage (early September). Experimental treatments included T1 and T2: amino acids (0.5 and 0.1 g L⁻¹), T3 and T4: seaweed (0.25 and 0.5 g L⁻¹), T5 and T6: micronutrients (0.1 and 1.5 g L⁻¹), T7: combined treatment of amino acid 0.1 g L⁻¹ with seaweed 0.5 g L⁻¹, T8: combined treatment of amino acid 0.1 g L⁻¹ with micronutrients 1.5 g L⁻¹, T9: combined treatment of seaweed 0.5 g L⁻¹ with micronutrients 1.5 g L⁻¹, T10: amino acid 0.1 g L⁻¹ with seaweed (0.5 g L⁻¹) and micronutrients (1.5 g L⁻¹), and T11: control treatment (the foliar application using distilled water). All treatments were applied as foliar application twice during the growing season. The concentration of the treatments was same in both foliar applications. Foliar application was carried out on flower and fruit clusters and on the upper leaves. The organic fertilizers included an amino acid (Aminabon 50, Spain) and the seaweed (Pigmasw 100, Spain). The combination of zinc, iron, and manganese elements in EDTA form was used as micronutrients in Kare Combi (Turkey) as a complete fertilizer.

Biochemical assays

After applying the treatments, the studied traits were evaluated in the stage of complete ripening of fruit in the laboratory, Faculty of Agriculture, Islamic Azad University, Jahrom Branch, Iran. To examine the traits, 20 fruits per replication were randomly selected. An extract was prepared to measure biochemical traits. Then, 15 ml distilled water was added to 3 g of fruit flesh. The mixture was stored for 12 hrs, during which it was stirred to finally obtain a completely homogeneous solution (Mostofi et al., 2009).

Soluble tannin

To measure soluble tannin, 5 ml of tannin extract

was diluted with 20 ml of deionized water and 5 ml of the deionized folin solution was added and mixed. After 5mins, 2.5 ml of a saturated sodium carbonate solution was added. After 1-2hrs, the amount of light absorption of soluble was read at a wavelength of 760 nm with a spectrophotometer (Perkin-Elmer 5100 model), and the amount of soluble tannin was determined based on the standard curve of tannic acid concentration (Mostofi et al., 2009).

Antioxidant activity

The antioxidant activity of the extracts was evaluated using the measurement of free radical-scavenging activity of the stable radical 2,2-diphenyl-1-picryl Hydrazyl (DPPH), which is an unstable free radical. By receiving an electron or hydrogen radical, it was rendered a stable radical. All extracts were prepared in a concentration of 1000 µg ml⁻¹, and 0.1 mM DPPH was added to one ml of methanolic solution. After 30 mins at room temperature, the absorptions were read at 517 nm against the blank. Then the percentage of inhibition of free radicals was calculated using the following formula:

$$\%IP = (A \text{ control} - A \text{ sample}) / A \text{ control} \times 100$$

Where, % IP is the percentage of inhibition of free radicals (percentage of inhibition against free radicals), 'A control' is the control adsorption (containing 1 ml of methanol in 1 ml of DPPH solution) and 'A Sample' is the sample adsorption (containing different volumes of plant extracts (antioxidants), methanol and DPPH solution). In this test, Butylated hydroxytoluene and ascorbic acid were used as positive controls.

Total phenol

Total phenolic content was measured using a Folin-Ciocalteu reagent, and then 2.5 ml of 10% Folin-Ciocalteu reagent was added to 0.5 ml of the extracts. After 5 min, 2 ml of 5% sodium carbonate solution was added, and the mixture was placed in a dark place at room temperature for 30 mins. Then, the absorbance at 760 nm was read by a visible-ultraviolet spectrophotometer against the blank (Sadeghi et al., 2015b). Gallic acid was used as the standard to draw the calibration curve and the total phenolic content was explained as mg gallic acid per g extract.

Measurement of protein

One ml of the solution containing the equal volume of sodium carbonate and NaOH 0.5 N, 1% copper sulfate, and 2% potassium sodium tartrate was added to the sample, and then 3 ml of Folin-

Ciocalteu reagent was added. The samples were placed at 5 °C and the absorbance was read at 625 nm against the control. Then, the standard curve was plotted using bovine serum albumin and the protein content was calculated (Lowry et al., 1951).

Total flavonoid content

Aluminum chloride colorimetric method was used to measure total flavonoid content (Chang et al., 2003). 500 µl of the extract was diluted with 1.5 ml ethanol (10% ethanol), 0.1 mL aluminum chloride, 0.1 ml potassium acetate 1 M, and 2.8 ml distilled water. Then, solutions were placed at room temperature for 30 mins. The absorbance was read at 415 nm with a visible-ultraviolet spectrophotometer. Quercetin was used to draw the standard curve, and total flavonoid content was explained as mg quercetin per g extracts.

Total carbohydrates

Total carbohydrates were measured using a Sigma-Aldrich kit (catalog code MAK104, Germany) based on the phenol-sulfuric acid reaction with polysaccharides. Polysaccharides are hydrolyzed and then converted to furfural, and combined with a dye complex to form a chromogen that is absorbed at 490 nm, and was measured by a spectrophotometer.

Measurement of mineral elements

Calcium (Ca)

For this purpose, the sample (20 mg) was dissolved in a solution of three acids, i.e. nitric acid, sulfuric acid, and perchloric acid (1 HNO₃: 1 H₂SO₄: 5 HClO₄) at 80 °C. This continued until the appearance of a red steam. Then, the temperature was raised to more than 150 °C for 7 hrs to give a clear solution. Calcium content was measured by atomic absorption using a spectrophotometer at 422.7 nm and was expressed as grams per gram dry weight (Manganaris et al., 2007).

Phosphorus (P)

The phosphate content was determined by aqueous phosphorus molybdenum with the visible-UV spectrophotometer. The percentage of phosphate present was explained based on dye intensity reduced by the phospho-molybdate solution (Huang and Zhang, 2008).

Potassium (K)

Potassium was measured by a selective ionic electrode (Potassium Meter, spectroscopy technology) and the potassium concentration was determined as micrograms per gram based on the potassium chloride standard (Saradhulhat and

Paull, 2007).

Contents of magnesium, iron, manganese, copper, and zinc

A solution of HClO₄: 2 HNO₃ (20 ml) was added to the sample (2 g) and the mixture was placed at ~ 150 °C until a clear solution was obtained. Then, 5 ml hydrogen peroxide (H₂O₂) was added, and the sample was dissolved until it reached a final volume of 1 ml. The samples were placed at room temperature to cool. Then, 1% sulfuric acid (5 ml) was added and the final solution was filtered. After filtering, the solution was transferred to a 50 ml flask and volumized by adding deionized water. Finally, the concentrations of the elements in the sample were determined using an atomic absorption spectrometer; Shimadzu company model ATS 586 (Hassan et al., 2017).

Statistical analysis

To assess normality of the data, Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted by SPSS 20.0 software and, after assuring that the data were normal, the data were analyzed using combined analysis of variance as a randomized complete block design. Due to the insignificance of the year effect and the interaction of the year and treatment, the average data of the two years of the experiment were compared. The mean values were compared with the LSD test at 5% level and the graphs were drawn with Microsoft Excel (2007).

Results

According to the combined analysis of variance, there was no significant difference between the two years of study. The treatments had a significant influence on the assessed attributes. There was no significant interaction between year and treatment (Table 1). Due to the insignificance of the year effect and the interaction of the year and treatment, the average data of the two years of the experiment were compared.

Biochemical assays

The combined treatment T10 caused the maximum protein content (6.52) which was significantly different from all treatments and the minimum amount was observed in the control (5.52) which was not different from seaweed and micronutrients (5.71 and 5.67, respectively). By increasing the concentration of the treatments, the protein content also increased (Table 2).

Table 1. Combined analysis of variance in relation to the effect of different treatments on biochemical and mineral elements of Zahidi date fruit during two successive years

Traits	Mean of squares (M.S)						
	Replication D.F=2	Year (Y) D.F=1	Error of year D.F=2	Treatment (T) D.F=10	Y × T D.F=10	Error D.F=20	C.V %
Protein	0.006 ^{ns}	0.018 ^{ns}	0.019	0.248 ^{**}	0.031 ^{ns}	0.022	2.5
Reduced sugars	0.103 ^{ns}	1.102 ^{ns}	1.185	21.981 ^{**}	2.850 ^{ns}	1.241	1.9
Soluble tannin	0.66 ^{ns}	0.96 ^{ns}	1.02	13.34 ^{**}	1.56 ^{ns}	1.00	3.4
Total phenol	0.004 ^{ns}	0.205 ^{ns}	0.245	8.244 ^{**}	0.523 ^{ns}	0.303	3.2
Flavonoid	0.187 ^{ns}	0.825 ^{ns}	0.834	7.214 ^{**}	1.025 ^{ns}	0.915	3.2
Total carbohydrate	0.006 ^{ns}	0.859 ^{ns}	1.025	8.552 ^{**}	2.175 ^{ns}	1.444	1.9
†DPPH	0.72 ^{ns}	1.12 ^{ns}	1.08	16.12 ^{**}	2.12 ^{ns}	1.22	3.4
Phosphorous	224.8 ^{ns}	326.5 ^{ns}	385.4	1741.4 ^{**}	789.6 ^{ns}	423.1	3.8
Potassium	261.9 ^{ns}	536.2 ^{ns}	524.9	3448.9 ^{**}	968.3 ^{ns}	636.9	3.2
Calcium	1457.9 ^{ns}	528.9 ^{ns}	502.8	7132.9 ^{**}	859.4 ^{ns}	600.7	3.6
Magnesium	564.0 ^{ns}	326.5 ^{ns}	336.5	2363.3 ^{**}	745.2 ^{ns}	426.1	4.4
Iron	0.54 ^{ns}	0.18 ^{ns}	0.22	92.61 ^{**}	0.38 ^{ns}	0.21	1.2
Copper	0.025 ^{ns}	0.011 ^{ns}	0.017	0.052 ^{**}	0.018 ^{ns}	0.014	8.4
Zinc	0.64 ^{ns}	0.58 ^{ns}	0.54	16.67 ^{**}	0.96 ^{ns}	0.60	5.7
Manganese	0.017 ^{ns}	0.014 ^{ns}	0.011	1.927 ^{**}	0.028 ^{ns}	0.020	3.9

^{ns} and ^{**} not significant and significant at $p < 0.01$

†2,2-iphenyl-1-picrylhydrazil

The highest content of the reducing sugar occurred in response to the combined treatment T10 (63.25) and was not significantly different from the effect of seaweed and amino acids + seaweed and micronutrients + seaweed (62.85, 62.74, and 63.09, respectively). The lowest value belonged to the combined treatment of 0.1 g L⁻¹ amino acid and 1.5 g L⁻¹ micronutrients (57.40), which was not significantly different from the control and treatments of amino acid and micronutrients. Except for the control, in the other two treatments, the content of the reducing sugar increased with increasing the concentrations of the treatments (Table 2).

Micronutrients (1.5 g L⁻¹) caused the highest tannin contents (32.26) and was not significantly different from the control, seaweed and micronutrients (32.12, 30.74, and 32.32, respectively) and the combined treatment T10 had the lowest soluble tannin contents (26.34), which with amino acids + seaweed caused the best performance of fruit (Table 2). The highest amount of total phenol was observed in the combined treatment T10 (20.11) which was not significantly different from the effect of amino acids + seaweed (19.74), and the lowest amount (15.35) was observed in the control which was not significantly different from the micronutrient treatments. In this treatment, the phenolic content increased with increasing the concentration of the treatment (Table 2).

The combined treatment T10 (33.14) with the highest contents of flavonoids was not significantly different from seaweed +

micronutrients (32.85). The control (28.54) had the lowest total flavonoid content, which was not significantly different from the amino acid, seaweed, micronutrients, and amino acid + seaweed (28.70, 28.84, 29.25, 29.74, 30.17, and 29.89, respectively). With an increase in the concentration of two treatments of amino acid and seaweed, the flavonoid content increased (Table 2). The combination of 0.5 g L⁻¹ seaweed and 1.5 g L⁻¹ micronutrients caused the maximum amount of carbohydrates (65.77), which did not differ significantly from the effect of seaweed, amino acids + seaweed, and the combination treatment T10 (64.18, 65.26, 65.38, and 65.61, respectively). The contents of carbohydrates increased with an increase in the concentration of seaweed, and the minimum amount was observed in 0.1 g L⁻¹ micronutrients (61.74), which there was no difference with the treatments of the control, amino acid, 1.5 g L⁻¹ micronutrients, and amino acids + micronutrients (62.15, 61.90, 62.24, 62.30, and 62.36, respectively). By increasing the concentration of amino acids and micronutrients, the contents of carbohydrates also increased (Table 2).

The highest amount of DPPH was measured in the combined treatment T10, which was not significantly different from seaweed + micronutrients (36.82 and 35.02, respectively) and the lowest amount belonged to the control (28.72), which had no significant difference with the effect of amino acid and micronutrients (30.15 and 30.50) (Table 2).

Table 2. Mean comparison of the effects of different treatments on biochemical attributes of *Phoenix dactylifera* (cv. Zahidi)

Treatment	Attribute	Protein (%)	Reduced sugars (%)	Soluble tannin (mg g ⁻¹)	Total phenol (mg g ⁻¹)	†GAE	Flavonoid (mg g ⁻¹)	Total carbohydrate (%)	††DPPH (%)
(T1) Amino acid (AA) 0.5 g L ⁻¹		5.88def	57.47c	30.24cde	17.12c		28.70d	61.90c	30.15fg
(T2) AA 1.0 g L ⁻¹		6.08bcd	57.51c	28.60e	18.54b		28.84cd	62.24bc	31.22def
(T3) Algae 0.25 g L ⁻¹		5.71efg	59.69b	30.74abc	16.79c		29.25bcd	64.18ab	32.75cd
(T4) Algae 0.5 g L ⁻¹		5.78ef	62.85a	28.84de	17.25c		29.74bcd	65.26a	31.97def
(T5) Micronutrients (MN) 1 g L ⁻¹		5.67fg	57.44c	32.20ab	15.48d		30.17bcd	61.74c	30.50efg
(T6) MN 1.5 g L ⁻¹		5.79ef	57.55c	32.26a	15.60d		30.35bc	62.30bc	32.14de
(T7) AA 1.0 g L ⁻¹ + Algae 0.5 g L ⁻¹		6.14bc	62.74a	26.34f	19.74a		29.89bcd	65.38a	32.86cd
(T8) AA 1.0 g L ⁻¹ + MN 1.5 g L ⁻¹		6.22b	57.40c	30.32cd	18.70b		30.75b	62.36bc	34.17bc
(T9) Algae 0.5 g L ⁻¹ + MN 1.5 g L ⁻¹		5.94cde	63.09a	30.51bcd	17.14c		32.85a	65.77a	35.02ab
(T10) AA 1.0 g L ⁻¹ + Algae 0.5 g L ⁻¹ + MN 1.5 g L ⁻¹		6.52a	63.25a	26.46f	20.11a		33.14a	65.61a	36.82a
(T11) Control		5.52g	57.41c	32.12ab	15.35d		28.54d	62.15bc	28.72g

Means in each column with same letters are not different according to LSD test $p < 0.05$.

†Gallic acid equivalent

††2,2-diphenyl-1-picrylhydrazyl

The contents of minerals

The highest phosphorus content (580) was observed under the effect of T10 which did not differ significantly from the treatments of seaweed and seaweed + micronutrients (569 and 557). The lowest value (509) was observed 0.1 g L⁻¹ amino acid + 0.5 g L⁻¹ seaweed, which was not

significantly different from the treatment of the control, amino acid, seaweed, micronutrients, and amino acid + micronutrients (520, 514, 527, 541, 512, 524, and 528, respectively). By increasing the concentrations of amino acids and micronutrients, the phosphorus content also increased (Fig. 1).

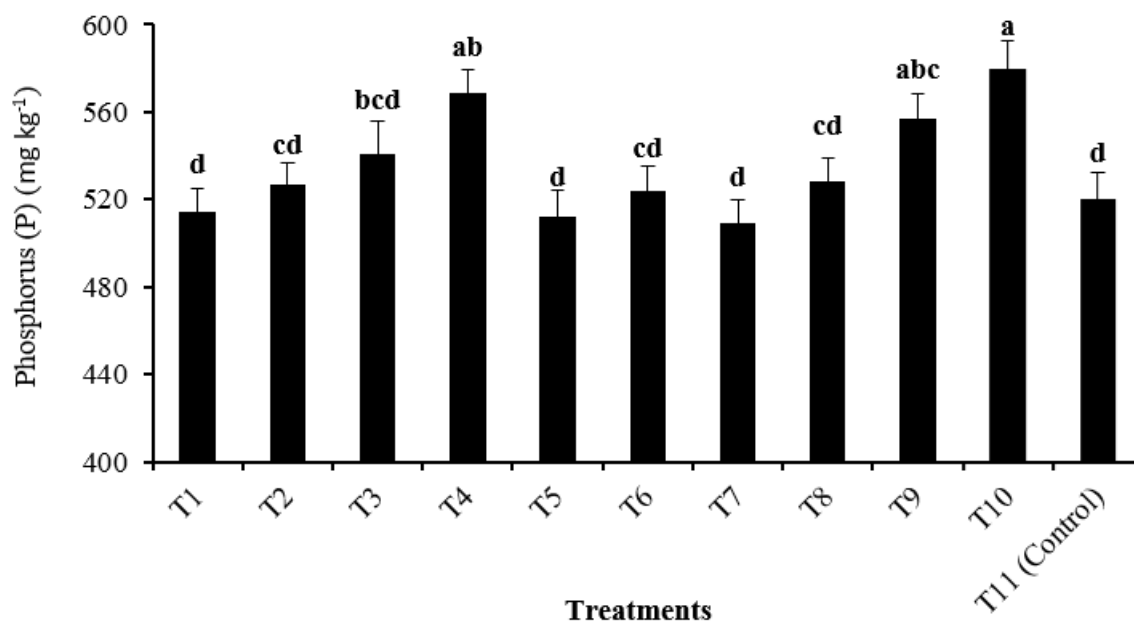


Fig. 1. Effect of different treatments on phosphorous (P) content in Zahidi date fruit.

The columns with same letters are not significantly different according to LSD test ($p < 0.05$).

T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

The combined treatment T10 caused the highest potassium content (846), which was not different from seaweed + micronutrients (832). The amino acid at 0.5 g L⁻¹ made the lowest potassium content (737) and was not significantly different from the treatments of the control, amino acid, seaweed, and micronutrients (745, 751, 774, and 769, respectively). By increasing the concentration of amino acid, the potassium content also increased (Fig. 2).

Under the effect of the combined treatment T10,

the highest calcium content (784) was recorded, and it was significantly different from the effects of other treatments. The control treatment caused the lowest calcium content (616) and did not differ from the treatments of amino acid, seaweed, and micronutrients (635, 650, 640, and 645, respectively). By increasing the concentration of amino acid, the calcium content increased (Fig. 3).

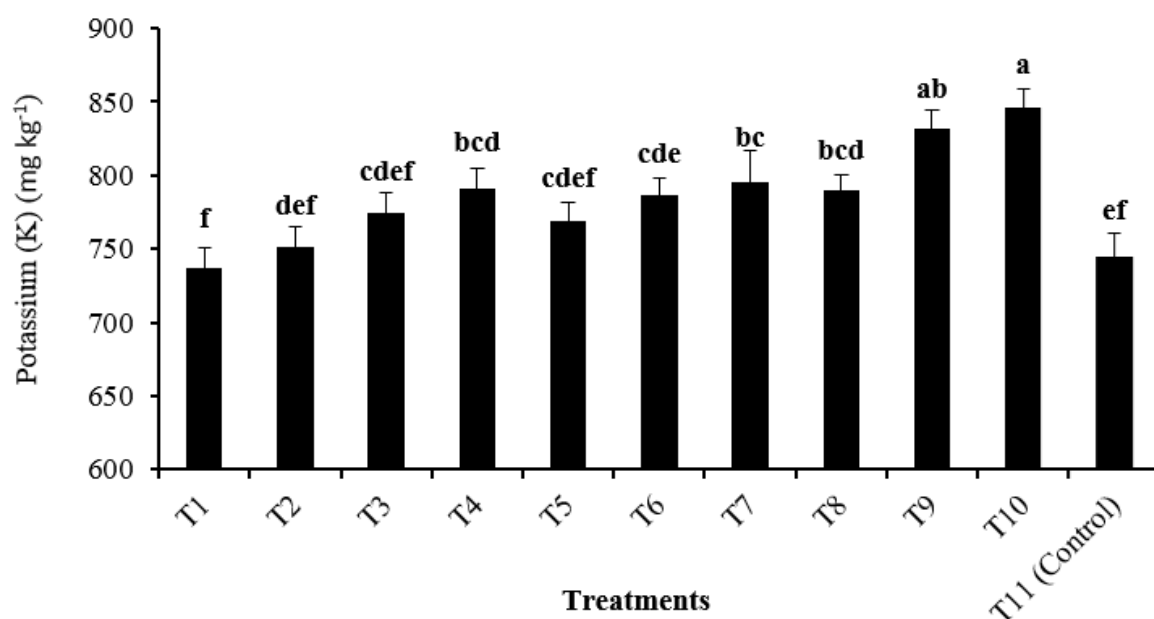


Fig. 2. Effect of different treatments on potassium (K) content in Zahidi date fruit.

The columns with same letters are not significantly different according to LSD test ($p < 0.05$).

T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

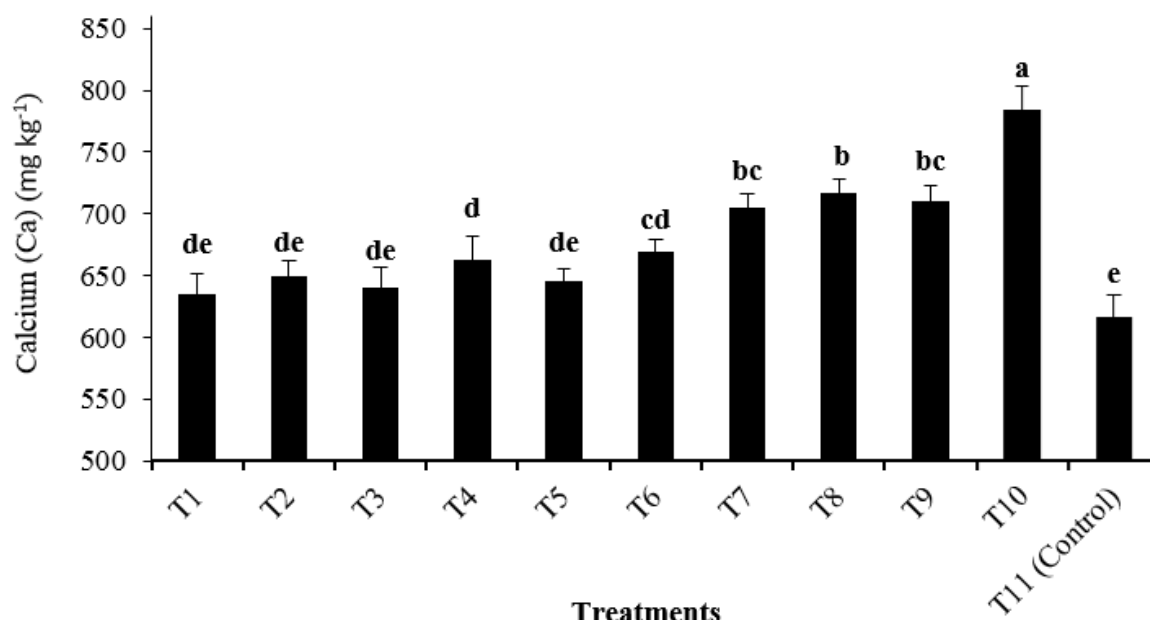


Fig. 3. Effect of different treatments on calcium (Ca) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

The highest magnesium content (514) was observed in the combined treatment T10, although there were no significant differences compared with the effect of amino acids + seaweed and amino acids + micronutrients (504 and 483). The lowest value (426) was observed under the effects of 0.1 g L⁻¹ micronutrients,

which was not significantly different from the treatments of the control, amino acids, seaweed, and micronutrients (437, 448, 454, and 440, respectively). By increasing the concentration of micronutrients, the magnesium content increased (Fig. 4).

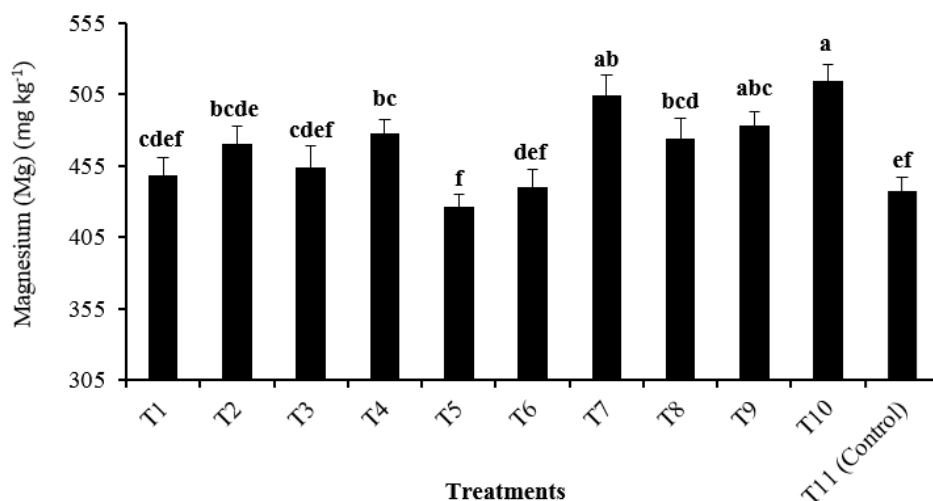


Fig. 4. Effect of different treatments on magnesium (Mg) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

The combined treatment T10 caused the highest iron content (47.1), and was significantly different from all other treatments. Seaweed at a

concentration of 0.25 g L⁻¹ caused the lowest iron content (30.8), which did not differ significantly from the amino acid treatment (31.5) (Fig. 5).

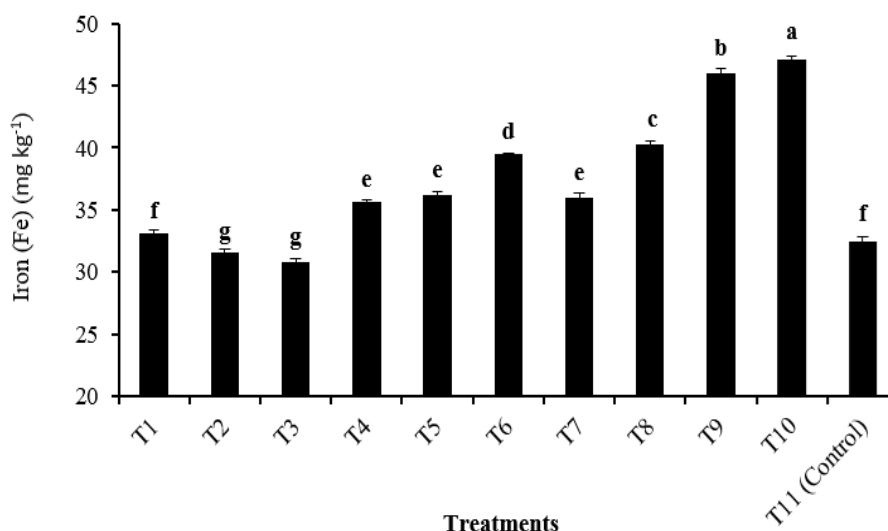


Fig. 5. Effect of different treatments on iron (Fe) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

The highest copper content (1.63) was observed under the effect of 0.1 g L⁻¹ amino acid + 1.5 g L⁻¹ micronutrients, and there was no significant difference with the effects of amino acid, amino acid + seaweed, and combined treatment T10 (1.52, 1.60 and 1.58). The lowest value belonged

to 1.5 g L⁻¹ micronutrients (1.26), which was not different from the treatments of the control, amino acids, seaweed, micronutrients, and seaweed + micronutrients (1.35, 1.40, 1.30, 1.37, 1.31, and 1.36). By increasing the concentration of seaweed, the copper content increased (Fig. 6).

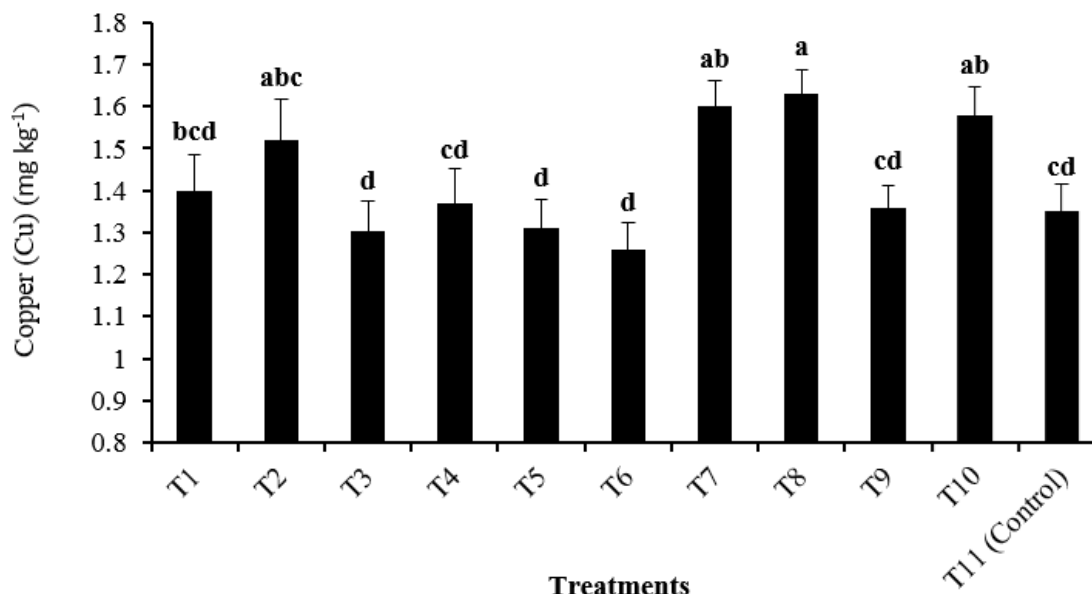


Fig. 6. Effect of different treatments on copper (Cu) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

The combined treatment T10 (16.61) caused the highest zinc content and there were no significant differences with the effects of micronutrients, amino acid + micronutrients, and seaweed + micronutrients (16.02, 16.24, and 16.39). The control had the lowest zinc content (11.23), which was not significantly different from the treatments of amino acid, seaweed, and amino acid + seaweed (11.30, 11.38, 11.55, 11.86, and 11.97, respectively). By increasing the concentrations of amino acid and seaweed, the

zinc content increased (Fig. 7).

The combined treatment T10 caused the maximum manganese content (4.95) and was not significantly different from the effects of seaweed + micronutrients (4.82). The minimum value was observed in the control (2.84) and there were no significant differences compared with the effects of amino acid, seaweed, and amino acid + seaweed (2.91, 2.94, 3.13, and 3.08, respectively). By increasing the concentration of amino acid, the manganese content increased (Fig. 8).

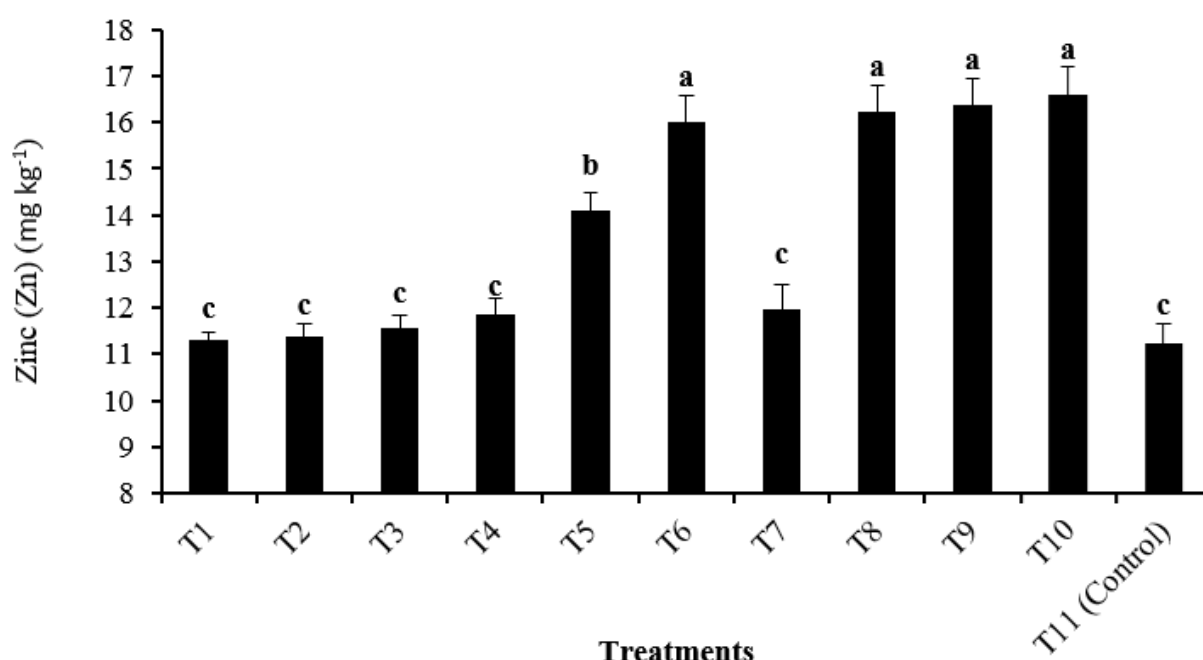


Fig. 7. Effect of different treatments on zinc (Zn) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

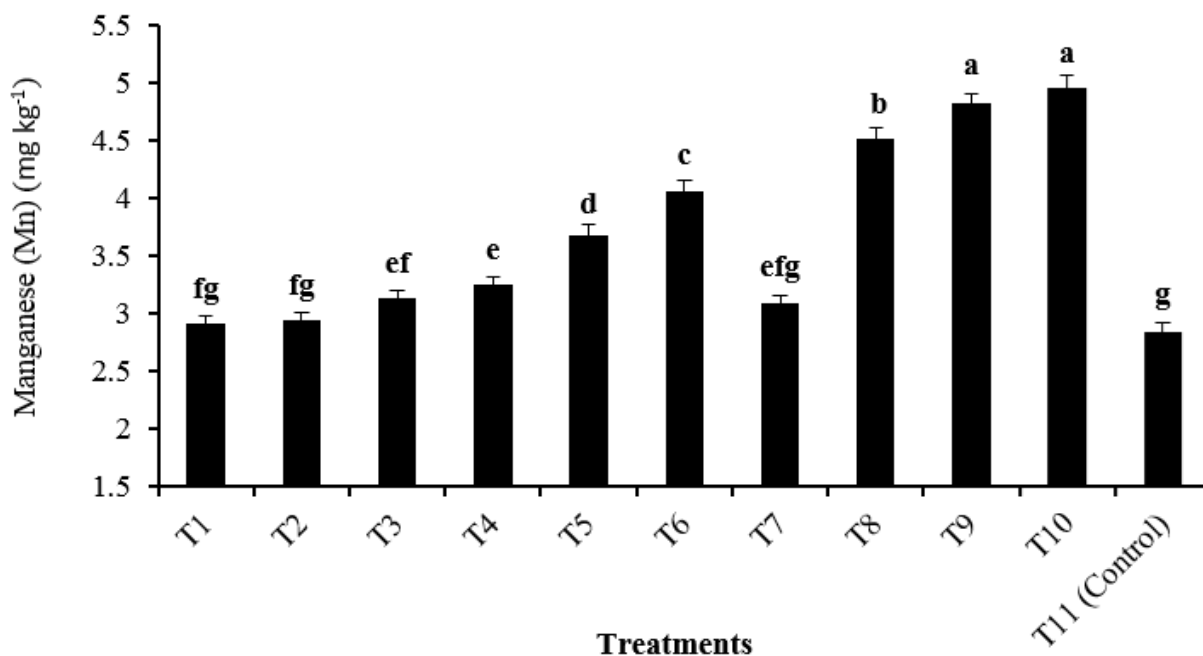


Fig. 8. Effect of different treatments on manganese (Mn) content in Zahidi date fruit. The columns with same letters are not significantly different according to LSD test ($p < 0.05$). T1, T2: amino acid (0.5, and 1.0 g L⁻¹); T3, T4: seaweed (0.25, and 0.5 g L⁻¹); T5, T6: micronutrients (1.0, and 1.5 g L⁻¹); T7: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹; T8: amino acid 1.0 g L⁻¹ + micronutrients 1.5 g L⁻¹; T9: seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T10: amino acid 1.0 g L⁻¹ + seaweed 0.5 g L⁻¹ + micronutrients 1.5 g L⁻¹; T11: control treatment (distilled water spraying).

Discussion

The results showed that the treatments had significant effects on protein content. The most important vital compounds for plant cells are proteins, the long and heavy molecules that form peptide chains by binding amino acids to each other. About 20 types of amino acids and 2 types of amides are involved in the process of protein production in plants (Jander and Jashi, 2010). Proteins (enzymes) control all plant functions. Proteins are synthesized directly by amino acids. Therefore, the amino acid treatment plays a direct role in increasing proteins. Seaweeds contain amino acids and have a positive effect on their synthesis. Components of some proteins and most enzymes, iron and zinc, are also effective in increasing the level of proteins. Previous research indicated this positive effect on potatoes (Dadashzadeh and Farajzadeh Memari-Tabrizi, 2020). Usually, the amount of reducing sugars in the fruit ripening stage is more than all other growth stages because as the fruit matures (aging), the speed of respiration and metabolism increases. Amino acids affect the amount of degrading enzymes such as pectinase, polygalacturonase, etc. These enzymes convert amino acids and organic acids to sugar, thereby increasing the sugar content. Hassanzadeh et al. (2015) reported this positive effect on pomegranate. The level of soluble tannins in date fruits is quite the opposite of reducing sugar content; when one increases, the amount of the other decreases, which justifies the effects of treatments and results in the current research. Flavonoids, anthocyanins, and phenolic acids in date extract are phenolic compounds and secondary metabolites of photosynthesis. According to the results of the current research, it was found that by increasing phenolic compounds and flavonoids, antioxidant properties also increased. The level of phenols and phenolic compounds in plants usually depends on the type of nutrient, protein production, photosynthesis, and enzyme activities. Research treatments have been able to increase, directly and indirectly, all plant functions, including structural, enzymatic, metabolic, and transferal, thereby increasing the biomass (chlorophyll) and leaf area index, which ultimately led to increased photosynthesis and production. Many studies have reported on the positive effects of this treatments on phenol, flavonoids, and antioxidant capacity of amino acids in basil (Fallahi et al., 2018), increasing the total phenol content in tomatoes (Moradi et al., 2020) and coriander (Aminifard et al., 2019). The carbohydrates in dates are formed from sugar,

and the drier the fruit, the higher the amount. Seaweeds contain monosaccharides and polysaccharides. They can directly increase carbohydrate production and indirectly increase leaf chlorophyll content. Moreover, they can stimulate photosynthesis and positively affect the biosynthesis of polysaccharides. Leaves are the most important reservoirs of carbohydrates. By affecting carbohydrate metabolism and storage, micro-elements such as iron and zinc become a part of the structure of enzymes and regulate their activity, thereby increasing chlorophyll content and leaf area index to improve carbohydrate storage and photosynthetic efficiency.

The amino acid treatment improved the potassium content in two ways in this study. One of the roles of amino acids is to regulate the stoma activity, which is done by potassium pumps and controlled by various factors, including the concentration of salts. This treatment could increase the potassium content by absorbing potassium for stomatal conductance. Another significant characteristic of amino acids is their chelating power and high mobility in plants, which facilitates the absorption and transfer of elements such as potassium in plants. Haghghi and Mozafarian (2015) confirmed these effects in greenhouse tomatoes. Seaweed extract also directly increased the amount of this element with micro- and macro-elements such as potassium, amino acids, and a variety of hormones, and this effect has been reported in almond leaves (Sa et al., 2015). Since chlorophyll is present in the core of magnesium, and amino acid and seaweed treatments can significantly affect the chlorophyll content, it seems that these treatments can affect plants and assist in their ability to absorb the amount of this substance, which leads to the increase in its amount in the fruit significantly. In addition, the seaweed extract contains magnesium. In the present study, the treatments affected positively (both directly and indirectly) the levels of iron, zinc, and manganese. All three treatments in this study, which included micro-elements (iron, zinc, and manganese), amino acids (iron and zinc), and seaweed (iron, cobalt, magnesium, molybdenum, zinc, and nickel), contained different amounts of these elements that directly affected the concentration of these elements. Furthermore, the amino acid treatment, with its chelating power, transferred these elements to the plant and increased its amount. Previous studies have reported the positive effect of using iron and zinc elements on apples (cv. Granny Smith) (Dehghani-Poudeh et al., 2019), quince seedlings (Mir Abdolbaghi, 2020), and tomatoes, using the hydroponic

system (Moradi et al., 2020a), as well as marigold flowers using the hydroponic system (Izadi et al., 2020). In the present research, the combined treatment (amino acids at 1.0 g L⁻¹ + micro-elements at 1.5 g L⁻¹) caused the highest copper content. The amino acid treatment at 1.0 g L⁻¹ had the most significant effect (in all treatments with amino acids at 1.0 g L⁻¹ which did not differ significantly with the maximum treatment). This performance was due to the chelating power of the amino acids. In this study, the combined treatment T10 had the highest phosphorus content and, by increasing the activity of soil enzymes such as phosphatase enzyme, the amino acid treatment increased the access of plants to phosphorus. The seaweed treatment contained macro-elements such as phosphorus and potassium, which directly increased the phosphorus concentration. Calcium was the most important mineral that affected fruit quality. The strongest effects of calcium on the cytoplasm were the regulation of plant respiratory activity, as well as cell wall and plasma membrane stability. Calcium usually moves slowly from leaves to fruits through the phloem. The low biological activity of calcium and high biological activity of amino acids when entering plant cells are necessary for the transfer and activity of calcium in plants (Tan, 1986). Therefore, research treatments could have a positive effect on calcium levels.

Conclusion

According to previous studies, it was found that organic fertilizers, including amino acids, seaweed, and micro-elements, could have significant, positive effects on biochemical traits and mineral content. Given that plant access to calcareous soils is limited to nutrient uptake, the foliar application of these organic fertilizers can lead to higher yields, resulting in better quantity and quality, especially for the purpose of export and international marketing. In general, the combined treatment T10 caused the best biochemical properties and the highest mineral contents. It is recommended that future studies evaluate other compounds, with different concentrations, in other growth stages (even before pollination) and with different frequencies of foliar application on Zahidi dates.

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Conflict of interest

The authors indicate no conflict of interest for this work.

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