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The effect of spraying with marine algae extract and adding Humax on growth traits of the roots of two varieties of pomegranate seedlings

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Abstract

The study was conducted for the period from 20/3 to 20/9/2023 to study the Effect of spraying marine algae extract and adding Humax on the growth traits of the roots of two varieties of pomegranate seedlings. The experiment was implemented relying on the completely randomized block design (RCBD) as a factorial experiment of three factors: the first was pomegranate varieties (Wonderful and Slimi), the second was marine algae extract with three concentrations (0, 1.5, and 3 ml L^{-1}), and the third was Humax with three concentrations (0, 0.25, and 0.50 g L^{-1}), comprising three replicates of each. On September 20 2023 measurements were taken and the results were analyzed according to the statistical design used ,and the averages were compared according to the selection of the least significant difference and at the level of probability ,0.05 and the most important results reached: The variety Wonderful was significantly superior to the Slimi variety in most root traits studied except the root diameter trait. The treatment of the marine algae extract at a concentration of 3ml L^{-1} showed significant superiority for all root traits studied, The Humax treatment at a concentration of 0.50 g L^{-1} showed significant superiority for all the root traits studied. The binary interaction between the varieties and the marine algae extract significantly affected all studied traits, as the variety Wonderful, when treated with 3ml L^{-1} increased in most root traits, while cultivar Slimi recoded highest average in the root diameter. The binary interaction between the varieties and Humax was significantly effective; when treating the variety Wonderful with 0.50 g L^{-1} of Humax increased all root traits significantly. The binary interaction between marine algae extract and Humax significantly affected all root traits. Treating seedlings with 3ml L^{-1} of marine algae extract and 0.50g L^{-1} of Humax.

Keywords: pomegranate, marine algae extract, Humax

Introduction

Pomegranate *Punica granatum* L. belongs to the Punicaceae family. Trees and fruits have been well-known for their benefits since ancient times. They were even planted in the hanging gardens of Babylon and depicted on Pharaonic tombs. The flowers of the pomegranate have been called Jalnar [1]. It is believed that the original homeland of pomegranate is Iran and northwest India, and it is commercially grown widely in some countries worldwide. More than 23 varieties are grown in Iraq, the most important of which is the Wonderful, which is distinguished by its production abundance, dark red color, very large-size fruits, and a sour taste; and the variety Slimi, which is characterized by large round-shaped fruits, a green skin tinged with red, and a sweet-sour taste [2]. The number of fruit trees is estimated at approximately 6,495,705 trees in Iraq, with the average production per tree of 2.37kg, and the total production is approximately 242,671t annually [3]. The economic importance of pomegranates comes from their early fruiting in the third year of cultivation and the delayed fruit maturity, which extends to late summer until the beginning or mid-winter when the presence of other fruits except citrus decreases. Pomegranate peels are used in leather tanning because they contain tannin, also used to treat diarrhea. The fruits also contain some medicinal substances, such as anthocyanin and phenolic substances, which have proven effective as antioxidants and inhibitors of several pathogens [4]. Producing healthy seedlings is crucial for expanding fruit cultivation, developing it further, and increasing yields. Therefore, developed countries use methods that help speed up the seedlings' arrival to the appropriate size, including spraying the shoots with plant extracts, marine algae extract. Foliar nourishment is more economical in using a small amount of nutrients that are applied at different plant stages in appropriate concentrations and in a way that provides the plant's nutrient requirements compared to large quantities of them that are added into the soil [5]. Marine algae extract is considered an essential nutrient for the plant and its growth by increasing the formation of organic matter and increasing the permeability of the cell wall in the plant's root cells, thus allowing nutrients to enter the plant, leading to improving the root growth traits [6]. Recent interest in the food product quality and its safety from pesticide residues and chemical fertilizers has led to relying on fertilizing with organic fertilizers due to their effect in improving the physical and chemical characteristics of soil by reducing the pH value and thus increasing the availability of essential elements due to the organic acids (humic and fulvic), as they work to chelate the elements in complex formulas, which is reflected in improving the root growth traits of pomegranate seedlings [7,8] found that spraying marine algae extract at a concentration of 4 ml L^{-1} and adding humic acid at a concentration of 4 ml L^{-1} to kaki seedlings gave the highest average root length. [9] concluded that adding humic acid to fig seedlings at a concentration of 50 ml L^{-1} resulted in the highest average root length and root dry weight. For the lack of studies on the use of marine algae extract and Humax in the growth of pomegranate seedlings and to reduce the time, effort, and cost of obtaining seedlings suitable for transfer to a permanent farm, the study

aimed to investigate the best type and concentration of marine algae extract and Humax and their effect in improving the root growth traits of pomegranate seedlings.

Materials and Methods

The study was implemented in the plant canopy at the Department of Horticulture and Landscape Engineering, College of Agriculture/ University of Kerbala for the period from 20/3 to 20/9/2023 to study the Effect of spraying with marine algae extract and adding Humax on growth traits of the roots of two varieties of pomegranate seedlings. From seedlings grown in sandy soil in black plastic bags made of polyethylene with a capacity of 1.25 kg, 270 seedlings, as homogeneous as possible in size and vegetative growth, aged ten months, were selected. They were transferred on 10/3/2023 to pots capacitate 10 kg soil. The experiment was designed as factorial based on the randomized complete block design (RCBD) of three factors; the first factor was the pomegranate varieties (Wonderful and Slimi), and the second and third factors were marine algae extract and Humax fertilizer, within three replicates, contained 90 seedlings in each, distributed to 5 seedlings per experimental unit. The seedlings were sprayed four times monthly, starting from March 20, 2023, using a 2-liter hand sprinkler. With each concentration, 1ml of detergent, as a diffuser (instead of the Tween-20), was added to reduce the surface tension of the water molecules to get the vegetative plant parts completely wet. The seedlings were sprayed with marine algae extract Algazone as a foliar fertilizer at three concentrations (0, 1.5, 3 ml L^{-1}) early in the morning. The control treatment was sprayed with distilled water and detergent after irrigating the seedlings a day before the spraying date in order to increase the efficiency of the plant in absorbing the sprayed material because moisture has a vital role in the process of swelling the guard cells and opening the stomata, in addition to the fact that watering before spraying reduces the concentration of solutes in the leaf cells, and increases the penetration of ions of the spray solution into them [10]. The following morning, Humax was added at three concentrations (0, 0.25, 0.50 g L^{-1}) five times with a month interval between one addition and the next, starting on 3/21/2023. All service practices performed, including irrigation and weeding, were performed equally and whenever needed.

Statistical analysis

The The experiment was designed according to the design of the complete random sectors (R.C.B.D) as a factor experiment with three factors : the first was pomegranate varieties (Wonderful and Slimi), the second was marine algae extract with three concentrations (0, 1.5, and 3 ml L^{-1}), and the third was Humax with three concentrations (0, 0.25, and 0.50 g L^{-1}), and three repeaters containing 18 seedlings each and 5 seedlings per treatment ,thus bringing the number of seedlings to 270 seedlings. The measurements were taken at the end of the study on 9/20/2023. The results were compared relying upon the least significant difference test (L.S.D.) at the probability level of 0.05 [11], and the following characteristics were measured:

traits of root growth:

1- Root length (cm)

The roots were rinsed well with water to get rid of the mud; then, the length of the primary root was measured after uprooting the seedlings and separating the vegetative parts at the swollen crown area with a graduated measuring tape from the crown area near the surface of the soil to the farthest peak of the main root.

2- Root size (cm^3)

The seedlings' root system was measured with a graduated cylinder with a known volume of water and according to the displacement law.

3- Root diameter (cm)

It was calculated according to the equation of [12]

$$D = 2 \times \sqrt{\frac{V}{L}} \times \pi$$

Since:

D: root diameter (cm)

V: root volume (cm^3)

L: root length (cm)

Π: Pi, mathematical constant number (22/7)

4- Root dray matter percentage (%)

It was measured after removing the seedlings from the pots for each experimental unit of the study treatments, separating the shoot system from the root system at the swollen crown area, and washing the roots with water. After the fresh root weight was measured, samples were placed in perforated paper bags in an oven at a temperature of 70° until the weight stabilized. Next, the dry weight of the roots and dry matter percentage was calculated as an average for each experimental unit as follows: [13].

$$Root\ dray\ \ matter\ percentage\ \ (\%) = \frac{root\ dry\ weight}{root\ freshweight} x100$$

Results and Discussion

Root length (cm)

The statistical analysis results in table 1, show a significant difference between the varieties, marine algae extract concentration, and Humax concentration in the average root length of seedlings. The variety Wonderful was superior in this trait, giving the highest average, amounting to 68.844 cm, compared to the variety Slimi, which recorded the lowest average, amounting to 62.267 cm, with an increase of 10.562%. Increasing the concentrations of marine algae extract led to an increase in this trait, as the treatment with 3 ml L^{-1} of marine algae extract gave the highest value of the root length, averaging 80.733 cm, achieving an increase of 60.114%, compared to the lowest average in the control treatment, reaching 50.422 cm. Increasing the Humax concentrations increased this trait, as the treatment 0.50g L^{-1} Humax gave the highest root

length average, reaching 70.600 cm. while, the lowest average was 60.589 cm in the control treatment, where the percentage of increase was 16.522%.

The binary interactions between the study factors significantly affected the studied trait. The variety Wonderful, treated with the marine algae at the concentration of 3 ml L^{-1}, gave the highest root length, averaging 83.600 cm, while the lowest average was 46.533 cm, recorded by the variety Slimi at the control treatment, exhibiting an increase of 79.657%. Treating the variety Wonderful variety, with a concentration of 0.50 g L^{-1} Humax, gave the highest root length, averaging 73.267 cm, outperforming the variety Slimi, without adding Humax, which gave the lowest root length average, amounting to 57.133 cm, an increase 28.239%. The interaction between marine algae extract and Humax affected the average of this trait, the treatment with 3ml L^{-1} of marine algae extract and the 0.50 gm L^{-1} gave the highest root length of 85,800 cm on average, while the lowest average was in the control treatment (no addition), reaching 45.267 cm, with the increasing percentage of 89.542%. The triple interaction effect significantly increased the average of this trait, as the Wonderful variety had a concentration of 3ml L^{-1} of marine algae extract and 0.50 g L^{-1} of Humax gave the highest root length average, reaching 87.800 cm., outperforming the treatment of the Slimi variety, without spraying with marine algae extracts or adding Humax, which gave the lowest average of the trait, of 41.000 cm an increase 114.146%.

Table (1): Effect of the variety, marine algae extract, Humax, and their interaction on the root length (cm).

Variety	Marine algae extract concentration (ml L^{-1})	Humax concentration (g L^{-1})			Variety x Marine algae extract
		0	0.25	0.50	
Wonderful	0	49.533	55.000	58.400	54.311
	1.5	64.200	68.067	73.600	68.622
	3	78.400	84.600	87.800	83.600
Slimi	0	41.000	45.200	53.400	46.533
	1.5	57.200	63.400	66.600	62.400
	3	73.200	76.600	83.800	77.867
$_{0.05}$L.S.D	0.314				0.181
	Humax effect mean	60.589	65.478	70.600	Variety effect mean
$_{0.05}$L.S.D	0.128				
Variety x Humax	Wonderful	64.044	69.222	73.267	68.844
	Slimi	57.133	61.733	67.933	62.267
$_{0.05}$L.S.D	0.181				0.104
Marine algae					Marine algae

extract concentration x Humax					extract concentration effect mean
	0	45.267	50.100	55.900	50.422
	1.5	60.700	65.733	70.100	65.511
	3	75.800	80.600	85.800	80.733
$_{0.05}$L.S.D	0.222				0.128

Root size (cm^3)

Table 2, illustrates the significant effect of the varieties, marine algae extract, and Humax and their interaction on the root size. The variety Wonderful was superior in this trait, giving the highest average, amounting to 27.778 cm^3, compared to the Slimi variety, which recorded the lowest average, amounting to 24.963 cm^3, an increase rate 11.276%. The results in the table also showed that increasing the concentrations of marine algae extract led to an increase in the root size average, as the treatment of the concentration 3ml L^{-1} gave the highest root size averaging of 36.389 cm^3, which was superior to the lowest average (16.222 cm^3) produced by the control treatment by 124.318%. Adding Humax at higher concentrations also increased this trait value, as the treatment of adding 0.50 g L^{-1} Humax gave the highest average, reaching 30.000 cm^3. While, the lowest average amounted to 22.889 cm^3 in the control treatment, with a difference of 31.067%. The binary interactions between the study factors significantly affected the average of this trait. The variety Wonderful, when treated with 3ml L^{-1} of marine algae extract, gave the highest root size average, reaching 37.556 cm^3, higher than the lowest average (14.889 cm^3), given by the variety Slimi treated with the control treatment, an increase 152.239%. The variety Wonderful, with a concentration of 0.50 g L^{-1} of Humax, gave the highest root size, averaging 31.778 cm^3. While, the lowest average was obtained from the variety Slimi without adding Humax, producing 21.889 cm^3 root size, differing from the interaction above by 45.177%. The interaction between the marine algae extract and Humax also significantly affected this trait, as the treatment of spraying the marine algae at the concentration of 3 ml L^{-1} and adding 0.50 g L^{-1} of Humax gave the highest root size averaging 40.333 cm^3, while the lowest average was 12.333 cm3 recorded by the control treatment (no addition) differing by 227.033% from the interaction above. The effect of the triple interaction was significant in increasing this trait average, as the Wonderful variety sprayed with a concentration of 3ml L^{-1} of marine algae accompanied with adding 0.50 g L^{-1} of Humax, gave the highest root size reaching 41.667 cm^3 on average. In contrast, without any treatment of marine algae or Humax, the variety Slimy gave the lowest average, amounting to 12.000 cm^3, with a difference of 228.941%.

Table (2): Effect of the variety, marine algae extract, Humax, and their interaction on the root size (cm^3)

Variety	Marine algae extract concentration (ml L^{-1})	Humax concentration (g L^{-1})			Variety x Marine algae extract
		0	0.25	0.50	
Wonderful	0	12.667	18.000	22.000	17.556
	1.5	25.000	28.000	31.667	28.222
	3	34.000	37.000	41.667	37.556
Slimi	0	12.000	15.000	17.667	14.889
	1.5	22.000	24.333	28.000	24.778
	3	31.667	35.000	39.000	35.222
L.S.D $_{0.05}$	0.791				0.457
Humax effect mean		22.889	26.222	30.000	Variety effect mean
L.S.D $_{0.05}$	0.323				
Variety x Humax	Wonderful	23.889	27.667	31.778	27.778
	Slimi	21.889	24.778	28.222	24.963
L.S.D $_{0.05}$	0.457				0.263
Marine algae extract concentration x Humax					Marine algae extract concentration effect mean
	0	12.333	16.500	19.833	16.222
	1.5	23.500	26.167	29.833	26.500
	3	32.833	36.000	40.333	36.389
L.S.D $_{0.05}$	0.559				0.323

Root diameter (cm)

The statically analyzed results in table 3, demonstrate an insignificant difference between the varieties in the root diameter, yet the other factors significantly affected the trait. The table shows that increasing the concentration of the marine algae to 3ml L^{-1} increased the root diameter to 2.377 cm on average, outperforming the control treatment, which recorded the lowest root diameter, averaging 1.998 cm, an increase 18.968%. Similarly, the increase in the Humax concentration to 0.50 g L^{-1} resulted in an increase in the root diameter, reaching 2.282 cm, higher than the control treatment of 2.127 cm an increase 7.301%. The binary interaction between the study factors af-

fected the trait significantly. The variety Slimy produced the highest root diameter when it was treated with the marine algae extract at the concentration of 3ml L^{-1}, reaching 2.381cm on average, exceeding the lowest average recorded by the interaction between the variety Wonderful and the control treatment (1.997cm) an increase 19.228%. The variety Wonderful when it was treated with Humax at the concentration of 0.50g L^{-1} gave the highest root diameter, averaging 2.313 cm, exceeding the lowest root diameter average, reaching 2.112, which was recorded by the same variety, Wonderful without Humax added, by 9.510%. The interaction between the marine algae extract and Humax also affected the root diameter, as the treatment of the 3ml L^{-1} of marine algae extract with 0.50g L^{-1} of Humax achieved the highest average of the trait, reaching 2.429 cm, which was higher than the lowest average recorded by control treatment (no addition) which gave 1.854cm by 30.980%. The triple interaction significantly affected the trait, treating the variety Wonderful with 3ml L^{-1} of the marine algae extract and 0.50g L^{-1} of Humax was superior in the root diameter, exhibiting 2.441 cm. In contrast, without treatment by any of them, this variety gave the lowest root diameter, reaching 1.792cm, differing from the treatment above by 36.216%.

Table (3): Effect of the variety, marine algae extract, Humax, and their interaction on the diameter (cm)

Variety	Marine algae extract concentration (ml L^{-1})	Humax concentration (g L^{-1})			Variety x Marine algae extract
		0	0.25	0.50	
Wonderful	0	1.792	2.027	2.174	1.997
	1.5	2.211	2.273	2.324	2.269
	3	2.333	2.344	2.441	2.372
Slimi	0	1.917	2.041	2.038	1.999
	1.5	2.176	2.195	2.297	2.223
	3	2.331	2.395	2.417	2.381
L.S.D 0.05	0.039				0.022
Humax effect mean		2.127	2.2126	2.2823	Variety effect mean
L.S.D 0.05	0.015				
Variety x Humax	Wonderful	2.112	2.214	2.313	2.213
	Slimi	2.141	2.210	2.251	2.201
L.S.D 0.05	0.022				N.S
Marine algae extract concentration x Humax					Marine algae extract concentration effect mean

	0	1.854	2.034	2.106	1.998
	1.5	2.194	2.234	2.310	2.246
	3	2.332	2.369	2.429	2.377
L.S.D $_{0.05}$	0.027				0.015

Root dry matter percentage (%)

Results in table 4, refer to the significant effect of the varieties, marine algae concentration, Humax concentration, and their interaction on the dry matter percentage in the root system. The variety Wonderful was superior, giving 46.786% root dry matter, higher than Slimi, which recorded 43.436% root dry matter by 7.712%. The table also showed that increasing the concentrations of marine algae extract increased this trait, as the treatment with 3ml L^{-1} of marine algae extract gave the highest average for this trait, amounting to 53.928%. In comparison, the lowest average was 36.116% in the control treatment, referring to an increase caused by the marine algae extract treatment by 49.318%. Increasing the concentrations of Humax caused an increase in the dry matter percentage in the root system. Treating the seedlings with 0.50 g L^{-1} of Humax gave the highest average, amounting to 47.904%, while the untreated seedlings of the control recorded the lowest dry matter percentage in the lowest average, amounting to 42.051%; thus, there was 13.918% increase for the Humax treated plants compared to untreated ones. The binary interactions between the three study factors significantly affected the average of this trait, as the Wonderful variety with a concentration of 3ml L^{-1} of the marine algae extract gave the highest average, amounting to 55.638%. while, the Slimi variety without adding marine algae extract gave the lowest average, amounting to 34.842%, increasing the treated plants to untreated ones by 59.686 %. Also, the variety Wonderful, treated with Humax at the concentration of 0.50g L^{-1}, gave the highest average of 49.096%. In contrast, the lowest average was obtained from the variety Slimi without adding Humax, recording 40.139%, with an increase for the treated plants of 22.314% over the untreated plants. There was an effect of the interaction between marine algae extract and Humax on this trait, as the treatment with 3ml L^{-1} of marine algae extract and 0.50g L^{-1} of Humax gave the highest average of dry matter percentage (56.653%), while the lowest average was given by the control treatment (no addition) reached 32.420%, as the treatment with both of them altogether increased the dry matter in the roots by 74.747%. The triple interaction significantly increased the average of this trait, as the Wonderful variety treated with the marine algae at the concentration of 3ml L^{-1} and Humax at the concentration of 0.50g L^{-1} gave the highest average, reaching 57.467%. In comparison, the variety Slimy without treatment by any of these materials gave the lowest average, amounting to 31.167%, differing by 84.384%.

Table (4): Effect of the variety, marine algae extract, Humax, and their interaction on dry matter percentage in the root system (%)

Variety	Marine algae extract concentration (ml L^{-1})	Humax concentration (g L^{-1})			Variety x Marine algae extract
		0	0.25	0.50	
Wonderful	0	33.673	38.320	40.173	37.389
	1.5	45.103	47.247	49.647	47.332
	3	53.110	56.337	57.467	55.638
Slimi	0	31.167	35.370	37.990	34.842
	1.5	40.100	43.330	46.310	43.247
	3	49.150	51.667	55.840	52.219
$_{0.05}$L.S.D	0.737				0.425
Humax effect mean		42.051	45.378	47.904	Variety effect mean
$_{0.05}$L.S.D	0.300				
Variety x Humax	Wonderful	43.962	47.301	49.096	46.786
	Slimi	40.139	43.456	46.713	43.436
$_{0.05}$L.S.D	0.425				0.245
Marine algae extract concentration x Humax					Marine algae extract concentration effect mean
	0	32.420	36.845	39.082	36.116
	1.5	42.602	45.288	47.978	45.289
	3	51.130	54.002	56.653	53.928
$_{0.05}$L.S.D	0.521				0.300

The results showed from the tables (1,2,3,4) that Wonderful was superior to Slimi in most studied traits, possibly due to the genetic variance between the two varieties. Marine algae extract has a significant effect on all the studied traits, and this may be due to its containing natural growth regulators that encourage root growth by increasing cell division and some amino acids and vitamins that help strengthen the root system [14]. The extract also contains nutrients necessary for the plant growth such as nitrogen, magnesium, and iron, and the role in building the chlorophyll molecule the magnesium atom represents the center of the chlorophyll which leads to an increase the efficiency of the photosynthesis process and accumulation of carbohydrates and their transfer to the roots[15], which increases root growth traits, it represents an increase in the length, volume, the diameter and dry weight of the root system and root branches and increase their surface area for absorption, this agreed with[8], Humax addition significantly affects the studied traits because it contains humic acid, which

stimulates root respiration and growth and increases the absorption of necessary elements from the soil, including manganese, phosphorus, potassium, and calcium. It also enhances the chemical and physical characteristics of the soil as it increases the exchange capacity and availability of some nutrients [16]. It also stimulates growth by increasing cell division, developing the root system, and increasing the percentage of dry matter. Humic acid has an effect similar to the hormone auxin, which encourages root growth [17]. Auxins improve root growth parameters by increasing the permeability of the cellular membranes of the roots, increasing their effectiveness in absorbing and increasing nutrient concentrations in the root system. This is reflected in enhancing root growth traits. These findings are consistent with those of Hussein [9].

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