

Response of Wonderful Pomegranate Cutting to Foliar Application of Humic Acid and Different Growth Medium

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Article history:

Received: 1 November 2022

Accepted: 12 December 2022

Published: 30 June 2023

Keywords: Growth medium, Pomegranate cuttings, Foliar application, Humic acid.

Abstract

The present study was conducted to evaluate the effect of different growing mediums {sand, peat moss, and mix (sand + peat moss 1:1 v/v)} and spraying with 0,100 and 150 mg L⁻¹ of humic acid on vegetative and root parameters of “Wonderful” pomegranate hardwood cuttings at Bakrejo nursery station, Sulaimania city, Iraq in 2021. They were planted in polyethylene black bags on 15-3-2021. Using randomized complete block design within the factorial experiments with three blocks. The results indicated that leaves, roots and shoots number, root wet weight, and root length were increased significantly in peat moss medium. Cuttings grown in a mixed medium showed a significant increase of leaf growth including leaf area, leaf dry weight, and chlorophyll content. In addition, spraying with 100 mgL⁻¹ of humic acid caused a significant increase in the root characteristics, and shoot number compared to the control. Furthermore, the maximum root growth was recorded in the interaction between peat moss medium and applying 100 mgL⁻¹ of humic acid compared to other treatments.

<https://dx.doi.org/10.52951/dasj.23150101>

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Introduction

Pomegranate (*Punica granatum* L.) is a major tree crop widely planted in Kurdistan-Iraq. It belongs to the Lythraceae family and is native to Iran (Chater, 2017; Mayi *et al.*, 2021). The pomegranate tree has extremely distributed in both tropical and subtropical regions. It is considered a tolerant crop to drought, salt and diseases. Thus, it can be cultivated such an alternative crop in countries suffering from plant diseases and climatic issues (Chater *et al.*, 2018). Generally, the pomegranate tree can be propagated by sexual propagation, and asexual propagation including cutting, air-layering, stool layering and grafting as well as micropropagation (Chandra and Babu, 2010). However, the most common and convenient method of pomegranate

propagation is stem cuttings in the world. Also, Cutting is the cheapest and fastest method able to produce a fully developed stronger tree (Kaur and Kaur, 2016; Sharma *et al.*, 2009). Chandra and Babu, (2010) stated that pomegranate cutting obtained from hardwood is better than semi-hardwood and softwood in terms of rooting growth.

Root stimulating of cuttings has been already a vital issue to study by agricultural researchers. Pomegranate rooting is directly affected by the growing medium (Manila *et al.*, 2017). Appropriate growing media should provide sufficient moisture, aeration, and nutrient for cutting. The optimum growing media varies depending on cutting type, season and plant species (Hartmann *et al.*, 1997). Sand and peat moss are common

growing mediums used to produce plant cutting. Sand formed from silicate compounds with low water retention is extremely poor in nutrients and the diameter of its particle is approximately 2 mm (Alikhani *et al.*, 2011). Walczak *et al.*, (2002) indicated that the dry matter content of sand comprises only 0.1% of organic matter and 99.9% of mineral matter. On the other hand, peat is organic matter formed from decomposed fibrous material and it is able to retain water ten times higher than its volume (Alikhani *et al.*, 2011). Humic substances are the most essential component of soil organic matter estimated roughly 60%. They are formed as a result of occurred numerous chemical, microbiological and physical conversions of plant and animal residues (Muscolo *et al.*, 2007). The components of humic substances are divided into fluvic acids, humin as well as humic acids, nevertheless humic acids are the most important segment of humic substances extensively consumed for plant growth development (Moshtaghi *et al.*, 2011).

Humic acid previously has been well-known as a substantial nutrient source for the plant. Also, recently it is well-established that it can induce plant growth through the enhancement of a lot of plant biochemical processes (Nardi *et al.*, 2002). Moshtaghi *et al.*, (2011) mentioned that humic acid is a plant cell promoter in the processes of photosynthesis, respiration and synthesis of protein because it acts as a hormone-like substance. The hormonal activity of humic acid is precisely confirmed by (Quaggiotti *et al.*, 2004) who stated that the role of humic acid is similar to auxin activities on plant development.

Spite of the fact that humic acid is often applied to the soil due to its favorable effects on the enhancement of micronutrient and macronutrient solubilization in soil, water holding capacity and microbial population of soil (Moshtaghi *et al.*, 2011). However, the positive influences of foliar application of humic acid on other types of

fruit seedlings already have been recorded by several researchers such as increasing shoot length and leaf area of apricot (Fathy and El-Shall, 2010). Significant effects were obtained, as it showed an increase in Olive seedling height, main shoot diameter, the number of leaves and leaf area compared to the comparison treatment (Aml *et al.*, 2011). A significant increase in leaf area, number of leaves, and leaf chlorophyll content of loquat seedlings (Al-Alalaf, 2012). Promoting shoot growth of the young olive plant (Fernandez-Escobar *et al.*, 1996), and enhancing the chlorophyll content of asparagus, in addition, it seems that foliar application is a more reasonable technique rather than soil application fertilizers in terms of reducing quantity employed, leaching and environment pollution (Tejada and Gonzalez, 2003).

Since a couple of decades ago, the wonderful variety has become a desirable cultivar commonly cultivated in Iraq because of its optimum physiological properties. The wonderful's fruit is big with a bright red color. Its arils also are vivid red, high juice, acidity and soluble solids content. Its seeds are too soft which results to be familiar as a seedless variety in Iraq (Sepulveda *et al.*, 2000). Abdulrahman *et al.*, (2021) investigated that wonderful grown in Iraq contains a good percentage of total sugars, anthocyanin, phenol, and ascorbic acid. That is why most plant nurseries in Iraq try to propagate wonderful variety more and more. Regarding this point, the main objective of the current study is to evaluate the effect of different growing mediums and humic acid concentrations on vegetative and root parameters of wonderful pomegranate cuttings.

Materials and Methods

The study was conducted at Bakrejo nursery station, Sulaimaniyah city, Iraq, in 2021. It is 760 meters above sea level. The "Wonderful" pomegranate cuttings were obtained from the hardwood part of trees

20-25 cm in length. They were planted in black bags of polyethylene black bags on 15-3-2021. Three different growing mediums were used in the present study including sand, peat moss and mix (sand + peat moss 1:1 v/v) as the first factor. The structure of the mediums was already analyzed before planting (table 1 and table 2). While the second factor is foliar applications of the commercial humic acid with three levels of humic acid (0,100 and 150 mg L⁻¹) sprayed on 30/6/2021 and repeated at the same concentrations on 30/7/2021.

The cuttings were cultivated in the wooden canopy and they were protected from the hot climate by providing a green cloth canopy around and above them. In addition, they were also irrigated with sprinklers during the plant's requirement.

Data collection and measurements

The measurements of the following parameters were recorded on 26/11/2021.

- 1- Leaves, roots, and shoots number: The whole leaves, roots, or shoots numbers per cutting were counted in each replicates.
- 2- Weight of wet leaf and root (g): The entire leaves or roots were weighted by digital balance.
- 3- Leaf dry weight: 10 leaves per cutting were taken randomly. The leaves were dried in the oven at 72 °C until the leaf weight was stabilized.
- 4- Leaf area (cm²): Image J protocol was used to measure leaf area (Glozer, 2008).
- 5- Chlorophyll content (SPAD): Chlorophyll content was measured with SPAD 502 chlorophyll meter.
- 6- Shoot and Root length (cm): The length of shoots or roots of cuttings were measured by measuring tape.

Table 1. Some chemical and physical properties of the growing mediums

Medium types	sand	silt	clay	text	PH	Ec	CaCo3	N	P	K
	mg.Kg ⁻¹ soil					dS.m ⁻¹	g.Kg ⁻¹ soil	mg.Kg ⁻¹ soil		
sand	825	50	125	Sandy loam	7.5	0.00025	280	1.52	1.0	1.4
peat moss	900	30	70	loamy sand	5.9	0.0033	10.0	4.88	4.8	17.2
Mix	850	50	100	sandy loam	7.2	0.0033	200	2.40	2.0	5.9

Table 2. Some soluble cations and anions of the growing mediums

soil types	Ca+	Mg+	Cl-
	Mmolc.L-1		
sand	0.6	0.56	43.7
Peat moss	10.7	37.7	12.2
mix	2.0	3.36	19.3

Data Analysis

Experimental design and statistical analysis: the Randomize Complete Block Design (RCBD) within the factorial experiment (two factors; Growth medium and humic acid concentrations) was used with three blocks. The collected data were submitted to the analysis of variance (ANOVA) by using (SAS 9.1 software). Mean comparisons were carried out by

using Duncan's multiple range test at 5% (Al-Mehmedi and Al-Mehmedi, 2012).

Result and Discussion

The result in table 3 indicated that pomegranate cuttings grown in peat moss produced the highest significant numbers of leaves than other mediums. In addition, leaf area, leaf dry weight and chlorophyll content significantly increased in a mixed

medium. While the non-significant difference was found in the wet leaf weight as a result of the effect of the growth in the different mediums.

On the other hand, foliar application of humic acid has not recorded any significant

effect on leaf number, wet and dry leaf weight, and leaf area. However, a significant decrease was found only in the chlorophyll content when applying 150 mgL⁻¹ of humic acid.

Table 3. The effect of growing mediums and humic acid on some vegetative parameters of pomegranate cutting

Treatments	Plant Parameters				
	Leaves number	Leaf Wet weight (gm)	Leaf dry weight (mg)	Leaf area (cm ²)	Chlorophyll content (SPAD)
Peat moss	592.289 a	2.246 a	32.586 ab	10.602 b	15.217 b
sand	342.000 c	2.328 a	26.431 b	10.518 b	15.473 b
mix	495.556 b	2.436 a	35.944 a	12.214 a	16.543 a
Humic acid (0 mg L ⁻¹)	479.311 a	2.430 a	30.960 a	11.172 a	16.076 a
Humic acid (100 mg L ⁻¹)	470.911 a	2.237 a	35.530 a	11.098 a	16.426 a
Humic acid (150 mg L ⁻¹)	479.622 a	2.342 a	28.471 a	11.064 a	14.732 b

Different letters in each column indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

Regarding to the interactions between growing mediums and humic acid (table 4), there was a significant difference between the interaction treatments in leaves numbers, leaf area and chlorophyll content except for wet and dry leaf weight. It was observed that untreated cutting with humic acid and

grown in a medium recorded the highest value of the leaf area (13.083 cm²) and chlorophyll content (18.647 SPAD). Also, the highest leaf number (609.800) was found in the untreated cuttings with humic acid and grown in peat moss.

Table 4. The effect of the interactions between growing mediums and humic acid on some vegetative parameters of pomegranate cutting

Soil types X Humic acid concentrations		Plant Parameters				
		Leaves number	Leaf wet weight (gm)	Leaf dry weight (mg)	Leaf area (cm ²)	Chlorophyll content (SPAD)
Peat moss	0 mg L ⁻¹	609.800 a	2.310 a	30.746 a	11.633 b	15.867 b
	100 mg L ⁻¹	584.667 a	2.277 a	38.879 a	11.333 bc	13.493 c
	150 mg L ⁻¹	582.400 a	2.150 a	28.135 a	8.840 d	16.290 b
Sand	0 mg L ⁻¹	361.867 c	2.463 a	28.692 a	8.800 d	13.713 c
	100 mg L ⁻¹	355.000 c	2.283 a	25.495 a	10.280 c	18.480 a
	150 mg L ⁻¹	309.133 c	2.237 a	25.105 a	8.840 d	14.227 c
Mix	0 mg L ⁻¹	466.267 a	2.517 a	33.443 a	13.083 a	18.647 a
	100 mg L ⁻¹	473.067 b	2.150 a	42.216 a	11.680 b	17.303 ab
	150 mg L ⁻¹	547.333 ab	2.640 a	32.173 a	11.880 b	13.680 c

Different letters in each column indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

The most shoots numbers were achieved at the cuttings growing in peat moss (36.156) shoots which is significantly superior to other growth mediums (table 5). While cuttings sprayed with 100 or 150 mgL⁻¹ of humic acid obtained (31.689 or

29.444) shoot respectively with significantly higher compared to only 24.911 shoots in non-treated cuttings. On the other hand, the sand medium produced the maximum shoot length compared to other mediums as well as non-treated cuttings with humic acid (0

mg L⁻¹) produced the longest shoot compared to cuttings sprayed with humic acid. The root parameters including root numbers, the weight of wet root and root length have been positively affected by

growing in peat moss medium than other growth mediums. Likewise, cuttings treated with 100 mg L⁻¹ of humic acid significantly increased the root characteristics more than other treatments.

Table 5. The effect of growing mediums and humic acid on some parameters of pomegranate cutting

Treatments	Plant Parameters				
	Shoots number	Shoot length (cm)	Roots number	Root wet weight (g)	Root length (cm)
Peat moss	36.156 a	21.324 c	20.422 a	6.978 a	34.333 a
sand	17.622 c	26.368 a	13.200 c	3.839 c	27.844 c
mix	31.644 b	23.546 b	17.756 b	6.562 b	30.444 b
Humic acid (0 mg L ⁻¹)	24.911 b	25.361 a	16.244 c	5.062 b	29.400 b
Humic acid (100mg L ⁻¹)	31.067 a	21.563 c	17.956 a	5.602 a	31.689 a
Humic acid (150 mg L ⁻¹)	29.444 a	24.313 b	17.178 b	5.714 a	31.533 b

Different letters in each column indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges.

Table 6 shows that interaction between peat moss medium and 100 mg L⁻¹ of humic acid improved the root characteristics of pomegranate cuttings significantly which recorded maximum roots number, root length and root wet weight compared to other interaction treatments. Conversely,

shoot length reached a highest value (29.023 cm) in the interaction between sand with 0 mg L⁻¹ of humic acid and the most shoot numbers were found in the interaction between peat moss and 0 mg L⁻¹ of humic acid (41.467).

Table 6. The effect of interactions between growing mediums and humic acid on some parameters of pomegranate cutting

Soil types X Humic acid concentrations		Plant parameters				
		Shoots number	Shoot length (cm)	Roots number	Root wet Weight (gm)	Root length (cm)
Peat moss	0 mg L ⁻¹	41.467 a	19.210 d	18.133 c	5.353 bc	31.467 c
	100 mg L ⁻¹	34.000 b	21.933 bc	22.667 a	7.847 a	36.333 a
	150 mg L ⁻¹	33.133 b	22.830 b	20.467 b	7.733 a	35.200 a
Sand	0 mg L ⁻¹	13.667 e	29.023 a	12.800 e	3.730 d	28.667 d
	100 mg L ⁻¹	25.467 c	21.420 c	13.867 d	3.937 d	28.333 d
	150 mg L ⁻¹	13.733 e	28.660 a	12.933 e	3.850 d	26.533 e
Mix	0 mg L ⁻¹	19.733 d	27.850 a	17.800 c	6.103 b	28.067 d
	100 mg L ⁻¹	33.733 b	21.337 c	17.333 c	5.023 c	30.400 c
	150 mg L ⁻¹	41.333 a	21.450 c	18.133 c	5.560 bc	32.867 b

Different letters in each column indicate a statistical difference ($p \leq 0.05$) between treatments according to Duncan's multiple ranges

The peat moss medium increased the leaves and shoots number and root characteristics. In addition, leaf area and chlorophyll content were significantly increased by the mixed medium. While the sand medium was no significant effect on all characteristics in the present study except shoot length. This situation refers to the role of peat moss in increasing water holding capacity and gradual nutrient release (Alikhani et al., 2011). The absence of a

significant effect of sand is connected to the low nutrient content of sand. Table 1 and table 2 show that the nitrogen, phosphor, potassium, calcium and magnesium content of sand are extremely lower than peat moss and mixed medium. Furthermore, the water holding capacity and aeration of sand are low and these factors play a vital role in the enhancement of root growth of cuttings (Rajkumar et al., 2017).The negative effect of the sand medium was also recorded on

root production of olive cuttings (Isfendiyaroglu et al., 2009) and pomegranate cuttings (Rajkumar et al., 2017). The result is in disagreement with the achieved result (Hassanein, 2013) in Ficus Hawaii cutting which found that sand medium was better than peat moss in terms of leave number and plant height.

Foliar application of humic acid showed a significant increase in all root characteristics and shoot numbers in cuttings treated with 100 mg L⁻¹ of humic acid. This positive effect could be correlated to the role of humic acid in promoting the processes of photosynthesis, respiration, and synthesis of protein in plants as well as the hormonal activity of humic acid as same as auxin activities (Moshtaghi et al., 2011; Quaggiotti et al., 2004). Gibberellin-like of humic acid was also verified by Pizzeghello et al., (2001). Moreover, humic acid has cytokinin activity and cytokinins promote cell division and shoot formation (Zhang and Ervin, 2004). Inducing root growth by humic acid is also found in Gerbera (Nikbakht et al., 2008). Zhang and Ervin (2004) showed HA has cytokinin activity.

Recording a maximum increase in root growth by the interactions between peat moss and 100 mgL⁻¹ of humic acid could be related to the effective role of humic acid in increasing nutrient uptake in peat moss media for pomegranate cutting and leads to further root development. According to katkat et al. (2009) the macronutrient including N, P, K and Ca as well as micronutrients such as Fe, Zn, and Mn significantly increased in wheat by foliar application of humic acid.

Conclusion

We can be concluded that peat moss medium is better than sand and mix (sand + peat moss) for pomegranate cutting growth and foliar application of humic acid at the level of 100 mg L⁻¹ is effective for enhancement of root growth and some vegetative characteristics of pomegranate

cuttings. Additionally, the interaction between peat moss and 100 mg L⁻¹ of humic acid is the best interaction for root growth compared to other treatments.

Conflict of Interest

We have no conflicts of interest to disclose.

Acknowledgements

This study was supported by the Technical College of Applied Sciences, Sulaimani Polytechnic University

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