Response of Carissa to Adding Humic Substances and Arginine Amino Acid

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Abstract

The study was conducted in one of the glasshouses of the College of Agriculture at the University of Anbar in the city of Ramadi, located in longitude and latitude 33 and 25, to investigate the effect of adding humic substances and spraying with arginine amino acid on the vegetative and root growth of the Carissa plant. The experiment included two factors. The first factor was the humic substance at four concentrations, which are 0, 1, 2, and 3 mg L-1; the second was the amino acid arginine at three concentrations, 0, 3, and 6 mg L-1. The experiment was designed using a Randomized Completely Block Design (R.C.B.D) with three replications. The study showed a significant improvement in the interaction treatment between the arginine acid treatment A2 and the organic material treatment H3 in the rates of leaf area, leaf number, plant height, carbohydrates, nitrogen, root surface area, and root length. It reached 827.000 cm2, 17.333 leaves plant-1, 15.667 cm, 4.690%, 2.667%, 46.937 cm2, and 11.300 cm, respectively.

Keywords: Carissa. Humic substances. arginine amino acid. Apocynaceae family. spraying

استجابة نبات الكاريسا لإضافة المواد الدبالية والحامض الاميني الارجنين

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المخلص:

نفذت الدراسة في احد البيوت الزجاجية التابعة لكلية الزراعة / جامعه الانبار، لدراسة تأثير اضافة المادة الدبالية والرش بالارجنين في النمو الخضري والجذري لنبات الكاريسيا. وتضمن التجربة عاملين العامل الاول هو المادة الدبالية بأربعة تراكيز وهي 0 ,1 ,2 ,3 ملغم لتر−1، والعامل الثاني هو الحامض الاميني الارجنين بتلات تراكيز 0 ,3 ,6 ملغم. لتر−1، وصممت التجربة باستخدام تصميم القطاعات العشوائية الكاملة (R.C.B.D) Randomized Complete Block Design بثلاث قطاعات وثلاث اصص للوحدة التجريبية الواحدة وبلغ كل قطاع 12 معامله وبعدد 108 سندانه لكافة القطاعات وتلخصت نتائج الدراسة بوجود افضل زيادة معنوية في معاملة التداخل بين معاملة حامض الارجنين A2 ومعاملة المادة الدبالية H3 في معدلات المساحة الورقية و عدد الاوراق و ارتفاع النبات والكلوروفيل والكاربوهيدرات والنيتروجين والمساحة السطحية للجذور و طول الجذر وبلغت 827.000 سم2و 17.333 ورقة .نبات1− و 15.667 سم و 1,390 ملغم غم −1 و 4.690 % و 2.667 % و 46.937 سم2 و 11.300 سم.

الكلمات المفتاحية: نبات الكاريسا. المواد الدبالية. الاحماض الامينية (الارجنين) العائلة الدفلية. الرش الورقي

Introduction

The *Carissa macrocarpa* plant belongs to the Apocynaceae family and is an evergreen shrub growing up to 3.0 meters tall. It has simple, dark green, oval-shaped leaves arranged alternately in pairs. The flowers are white and fragrant, appearing in summer. The fruits are edible, with a bright red color and oval shape. They can be used indoors after providing adequate ventilation and lighting. Carissa plants can be grown individually or in clusters and are used as hedges because they contain thorns (Kaunda, Zhang, 2017). Despite its beauty and numerous uses, this plant suffers from noticeable neglect, as it is not widely propagated or cultivated in governmental or private nurseries.

Amino acids significantly improve plant growth and are organic compounds (Ahmed and almohammedi, 2021). Arginine is one of these essential amino acids; the symbol R or Agr represents it. It is an organic compound with the molecular formula $C_6H_{14}O_2$. Arginine has various physiological roles, serving as a reservoir for protein as a precursor to polyamines and nitric oxide, enhancing plant growth (Shafeek *et al.*, 2012; Fayek et al., 2011). Arginine is considered an essential amino acid in urea formation and plays a vital role in polyamide formation, promoting plant vegetative growth, root formation, chlorophyll synthesis, and cell division stimulation (Nasibi *et al.*, 2013 and Abd-Elkader et al, 2020).

Humic substances are produced from plant residues and animal waste. Their importance lies in their readiness as fertilizers and their nutrient content, rich in carbon compounds. Humic substances are characterized by containing fulvic acid, humic acid, and humin, as well as sulfur, phosphorus, carbon, nitrogen, and oxygen. They form layers resembling clay plates in their organization and distribution, possessing negatively charged surfaces crucial for cation exchange processes. Humic compounds are crucial for plants as they enhance the availability of micronutrients, improve soil

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chemical and physical properties, increase nutrient release, and enhance cation exchange capacity. Moreover, they are cost-effective, readily available, and can yield good results in promoting plant growth. (Abbas, 2021).

The study aimed to Early production of Carissa saplings and reduce the time required for their production and investigate the effect of the amino acid (arginine) on the vegetative and root growth characteristics, as well as the chemical content of the plant and examine the impact of humic substance on the growth characteristics and chemical content of the Leaf.

Materials and Methods

The experiment was conducted at the college of Agriculture - University of Anbar, in the greenhouse belonging to the Department of Horticulture and Garden Landscape, to study the response of Carissa plants to spraying with the amino acid arginine and the addition of humic substances from October 3, 2023, to May 1, 2024. The plants were cultivated in plastic pots weighing 15 kg containing peat moss and vermiculite in a ratio of 2:1. One plant was planted in each pot.

Study factors

involved the addition of humic substances Produced by Libro Organic Materials Company A one-time ground addition was added at four concentrations: 0, 1,2 and 3. included Second factor spraying with the amino acid arginine at three concentrations: 0, 3, and 6 mg L^{-1}.

Humic substances were added as a soil amendment two weeks after transplanting the saplings into the experimental pots. Then, the addition of humic substances was repeated one month after the first addition, with the initial addition of humic substances occurring on February 1, 2024. Regarding the spraying with arginine, the plants were sprayed one week after the addition of humic substances and at each subsequent addition of humic substances. Therefore, the humic substance was added three times, and the arginine was sprayed three times. The spraying was carried out until complete wetting, achieved when droplets of the solution descended from the leaves in the early morning, using a manual sprayer with a capacity of 2 liters. This was done to avoid sunlight and prevent evaporation. Additionally, spraying was conducted after watering to prevent washing away of arginine from the foliage and humic substances from the soil. All precautions were taken during the spraying process, including maintaining separation between treatments to ensure spraying according to the concentration for each treatment. The greenhouse was covered with a layer of soil (river sand) beneath the planted pots to preserve the plant roots in case they grew out of the pots and to maintain moisture around the pots. recommendation was added to all plants at a concentration of 1 gram per liter once for all treatments. All necessary services, including watering and weeding, were carried out for all treatments whenever needed.

The experimental design employed in this study was a factorial design with two factors: the first factor had four concentrations of humic substances, while the second factor had three concentrations of arginine (mg L^{-1}). The experiment was conducted using a randomized complete block design (R.C.B.D), with a total of 12 treatments and three replications. Each experimental unit included three plant pots. Thus, the total number of pots for the experiment was 108. The means were analyzed using the Genstat program, and comparisons between means were made using the Least Significant Difference (L.S.D) test at a significance level of 0.05 (Al-Mohammadi and Al-Muhammadi, 2012).

Study Indices:

Leaf Area per Plant (cm^2 $leaf^{-1}$): The leaf area per plant was calculated using the ImageJ software installed on the computer. Five fully expanded leaves were selected from the previously chosen plants in each experimental unit, then scanned using a scanner and transferred to the software. The average area of a single leaf (cm^2) was extracted (O'neal *et al.*, 2002).

Total Leaf Number (leaf $plant^{-1}$): The total number of leaves per plant was calculated for each experimental unit, and the average value for each treatment was then calculated.

Plant Height (cm): The average plant height was measured using a measuring tape from the soil surface to the highest point at the stage of emergence of green shoots for each experimental unit.

Leaf Carbohydrate Percentage (%):

Preparation and Digestion of Plant Leaf Samples: The nutritional elements were determined using the method of Cresse and Parsons (1979) in the laboratory of the College of Agriculture/University of Anbar for Postgraduate Studies. Plant leaf samples were ground after drying using an electric grinder and weighed at 0.5 g per treatment, then placed in a volumetric flask for digestion. Sulfuric acid

(H_2SO_4) was added at a ratio of 3 ml, and concentrated perchloric acid at a ratio of 1 ml, and then left for 24 hours. Afterward, they were placed on a hot iron plate (Hot Plate) until the samples turned colorless after getting rid of gases and vapors. The solution was then cooled, transferred to a 50 ml volumetric flask, and the volume was completed with distilled water. Finally, they were stored in dark glass containers for nutrient estimation.

Nitrogen Percentage in Leaves (%): Nitrogen was estimated using the Kjeldahl method (Jones, 1973)

Root Surface Area (cm^2): Ten roots were taken from each treatment, the Digimizer scanner program was used

Root Length (cm): The lengths of ten roots were measured at the end of the experiment, and their average was extracted after taking random samples. A regular ruler was used to measure the length of the roots.

Results and Discussion:

Average Leaf Area (cm^2): The results in Table 1 showed that adding the humic substance at a concentration of 3 mg L^{-1} led to a significant increase in the average leaf area of Carissa plants (757.667 cm^2) compared to the control treatment (619.000 cm^2). The results also indicated that spraying arginine acid at a concentration of 6 mg L^{-1} on the plants resulted in a significant increase in the average leaf area (751.667 cm^2) compared to the control treatment, which had a lower average of 656.250 cm2 respectively. The results of Table 1 showed that adding the humic substance at a concentration of 3 mg L^{-1} combined with spraying arginine acid at a concentration of 6 mg L^{-1} led to a significant increase in the average leaf area of 827.000 cm^2 compared to the control treatment (611.333) cm^2 respectively.

Table 1. Effect of spraying with arginine, adding humic substances, and their interaction on the average leaf area (cm^2)

Humic	Arginine concentrations			Mean H
	A0	A1	A2	
H0	611.333	619.333	626.333	619.000
H1	634.000	645.333	753.667	677.667
H2	668.000	721.667	799.667	729.778
H3	711.667	734.333	827.000	757.667
Mean A	656.250	680.167	751.667	
LSD 0.05	H	A	H*A	
	14.613	12.655	25.310	

Rate of increase in the number of leaves (leaf $plant^{-1}$)

The results indicate that adding the humic substance at a concentration of 3 mg L-1 led to a significant increase in the number of leaves of Carissa plants, at 14.000 leaves $plant^{-1}$ compared to the control treatment, which had 10.111 leaves $plant^{-1}$ (Table 2). Similarly, the results showed that spraying arginine acid at a concentration of 6 mg L^{-1} on the plants resulted in a significant increase in the number of leaves at 14.083 leaves $plant^{-1}$ compared to the control treatment, which had a lower average of 10.167 leaves $plant^{-1}$. The results demonstrated that adding the humic substance at a concentration of 3 mg L^{-1} combined with spraying arginine acid at a concentration of 6 mg L^{-1} led to a significant increase in the number of plant leaves at 17.333 leaves $plant^{-1}$ compared to the average control treatment of 8.333 leaves $plant^{-1}$.

Table 2. Effect of spraying with arginine, adding humic substances, and their interaction on rate of increase in the number of leaves (leaf plant^{-1}

Humic	Arginine concentrations			Mean H
	A0	A1	A2	
H0	8.333	10.333	11.667	10.111
H1	9.667	12.000	13.000	11.556
H2	10.667	13.333	14.333	12.778
H3	12.000	12.667	17.333	14.000
Mean A	10.167	12.083	14.083	
LSD 0.05	H	A	H*A	
	0.769	0.666	0.666	

Plant height (cm)

The results in Table 3 revealed significant effect of humic substances, and arginine concentration on the plant height rate, but the interaction of the study factors was not significant. The treatment with humic substance H3 at a concentration of 3 mg L^{-1} significantly surpassed all other treatments in plant height (12.889 cm), while the control treatment showed the lowest plant height at 8.667 cm.

Regarding the arginine-treated plants, the treatment A2 at a concentration of 6 mg L^{-1} significantly outperformed in plant height at 12.500 cm, while the control treatment at 9.250 cm in plant height. However, there was no significant difference found in the interaction between the studied factors.

Table 3. Effect of spraying with arginine, adding humic substances, and their interaction on the average plant height (cm)

Humic	Arginine concentrations			Mean
	A0	A1	A2	
H0	7.667	8.333	10.000	8.667
H1	8.667	10.667	11.333	10.222
H2	10.000	11.333	13.000	11.444
H3	10.667	12.333	15.667	12.889
Mean	9.250	10.667	12.500	
LSD 0.05	H	A	H*A	
	0.750	0.649	N.S	

Leaf Carbohydrate content (%)

The results in Table 4 indicate a significant increase in the carbohydrate content in the leaves of Carissa plants with the addition of humic substance at a concentration of 3 mg L^{-1} at 6.077%, compared to the control treatment which recorded 5.240 %.

Similarly, the application of arginine at a concentration of 6 mg L^{-1} led to a significant increase in the carbohydrate content in the leaves at 5.898%, compared to the control treatment (5.383%). Furthermore, the combined treatment of humic substance at a concentration of 3 mg L^{-1} with arginine at a concentration of 6 mg L^{-1} resulted in a significant increase in the carbohydrate content in the leaves, at 6.637%, compared to the control treatment, which recorded 5.167%.

Table 4. Effect of spraying with arginine, adding humic substances, and their interaction on Leaf Carbohydrate content (%)

Humic	Arginine concentrations			Mean
	A0	A1	A2	
H0	5.167	5.230	5.323	5.240
H1	5.260	5.363	5.517	5.380
H2	5.373	5.637	6.113	5.708
H3	5.733	5.860	6.637	6.077
Mean	5.383	5.523	5.898	
LSD 0.05	H	A	H*A	
	0.052	0.045	0.090	

Nitrogen percentage content leaves (%)

The results in Table 5 indicate significant differences in the nitrogen content in the leaves at the individual factor level and in the interaction between factors. The treatment with humic substance H3 at a concentration of 3 mg L^{-1} showed a significant increase in the nitrogen content in the leaves compared to the other treatments, at 2.196%. In contrast, the control treatment recorded the lowest nitrogen content of 1.726%.

Similarly, the treatment with arginine A2 at a concentration of 6 mg L^{-1} showed a significant increase in the nitrogen content in the leaves compared to the control treatment at 2.127% and 1.825%, respectively. Furthermore, the combined treatment of humic substance at a concentration of 3 mg L^{-1} with arginine at a concentration of 6 mg L^{-1} resulted in a significant increase in the nitrogen content in the leaves at 2.667%, compared to the control treatment (1.630%).

Table 5. Effect of spraying with arginine, adding humic substances, and their interaction on Nitrogen Percentage leaves (%)

Humic	Arginine concentrations			Mean
	A0	A1	A2	
H0	1.630	1.750	1.797	1.726
H1	1.820	1.857	1.880	1.852
H2	1.910	1.963	2.163	2.012
H3	1.940	1.980	2.667	2.196
Mean	1.825	1.888	2.127	
LSD 0.05	H	A	H*A	
	0.032	0.028	0.055	

Root surface area (cm^2)

Results indicate that adding humic substance at a concentration of 3 mg L^{-1} led to a significant increase in root surface area for the Carissa plants at 41.037 cm^2 compared to the control treatment of 34.890 cm^2 (Table 6).

Similarly, the results showed that spraying arginine acid at a concentration of 6 mg L^{-1} on the plants also led to a significant increase in root surface area at 40.792 cm^2 compared to the control treatment, which recorded 34.227 cm^2. Furthermore, the results also demonstrated that adding humic substance at a concentration of 3 mg L^{-1}, combined with spraying arginine acid at 6 mg L^{-1}, significantly increased root surface area. It reached 46.937 cm^2 compared to the control treatment, which recorded 31.813 cm^2 respectively.

Table 6. Effect of spraying with arginine, adding humic substances, and their interaction on Root surface area (cm^2)

Humic	Arginine concentrations			Mean
	A0	A1	A2	
H0	31.813	34.940	37.917	34.890
H1	33.510	35.493	37.520	35.508
H2	34.930	36.603	40.793	37.442
H3	36.653	39.520	46.937	41.037
Mean	34.227	36.639	40.792	
LSD 0.05	H	A	H*A	
	0.492	0.4268	0.853	

The Root Lenth(cm):

Results from Table 7 indicate that adding humic substance at a concentration of 3 mg L^{-1} led to a significant increase in the most extended root length for Carissa plants, reaching 10.310 cm compared to the control treatment, which was 9.124 cm.

Similarly, the results showed that spraying arginine acid at a concentration of 6 mg L^{-1} on the plants also significantly increased the length of the longest root, reaching 10.422 cm compared to the control treatment, which recorded 9.035 cm, respectively. Furthermore, the results also demonstrated that adding humic substance at a concentration of 3 mg, combined with spraying arginine acid at a concentration of 6 mg per liter, significantly increased the length of the longest root. It reached 11.300 cm compared to the control treatment, which recorded 8.593 cm.

Table 7. Effect of spraying with arginine, adding humic substances, and their interaction on the Root Lenth (cm)

Humic	Arginine concentrations			Mean
	A0	A1	A2	
H0	8.593	9.273	9.507	9.124
H1	9.073	9.530	9.803	9.469
H2	9.143	9.650	11.077	9.957
H3	9.330	10.300	11.300	10.310
Mean	9.035	9.688	10.422	
LSD 0.05	H	A	H*A	
	0.118	0.102	0.205	

The results from Tables 1, 2, 3, 4, 5, 6, and 7 reveal that treatments with spraying arginine amino acid at a concentration of 6 mg L^{-1}, along with humic substance treatment at a concentration of 3 mg L^{-1}, have achieved a significant increase in the vegetative growth indicators of Carissa plants. This increase includes the average leaf area, leaf count, plant height, total chlorophyll in the leaves, carbohydrates, nitrogen, root surface area, and root length. The reason behind this is amino acids' vital role in carbon assimilation, as they serve as a primary source of essential nitrogen for building proteins and necessary enzymes in constructing plant cells. Consequently, this leads to increased cell division and elongation, ultimately resulting in increased plant height, branch count, and leaf count on the plant (Taiz and Zeiger, 2006). The increase in vegetative growth indicators may be attributed to the role of these amino acids in increasing the content of plant tissues of proteins through the assimilation and formation of proteins. (Shraida and Almohammadi, 2021).

This may be attributed to amino acids, which are considered a nitrogen source for building proteins and enzymes and providing energy that encourages vegetative and root growth (Nahed and Balbaa, 2007). Applying amino acids to plants has multiple effects, especially arginine, as it plays a crucial role in forming amino compounds. These compounds are known for participating in enzyme activity modulation, cell division, elongation, and plant growth (Ahmad *et al.*, 2010).

The effect of humic substance can be attributed to its content of oxygen, hydrogen, carbon, and nitrogen in varying proportions and with lightweight molecular weights when used for plants. It plays a fundamental role in plant nutrition, reflecting on the process of photosynthesis, protein synthesis, other substances, and respiration (Mousavi *et al.*, 2012). Additionally, humic substances increase the internal levels of plant hormones such as auxins and gibberellins by stimulating their synthesis or preventing their degradation (Alam *et al.*, 2012). Perhaps the reason for the increase in vegetative growth indicators and the chemical content of the plant is the humic substance's role in increasing the proportion of solid oxygen compounds in the cells. This is consistent with what was stated by (I.A. and Zeboon, 2024; and Mahdi, 2023)

Conclusions

We conclude from the experimental parameters that adding the humic substance at a concentration of 3 mg L^{-1} gave the highest rates in most vegetative, root, and chemical traits. From the experimental parameters, we conclude that Sapring the Arginine amino acid at a concentration of 6 mg L^{-1} gave the highest rates in most vegetative, root, and chemical traits. Response of the Carissa plant to the study factors.

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