

The effect of adding humic acid and bio-fertilizers on the chemical properties of date palm leaves, Barhi

Sajjad Abdul Hassan Abadi Al Taher*, Harith Mahmoud Aziz Al-Tamimi

Department of Horticulture and Landscape Engineering, College of Agriculture, University of Kerbala, Karbala, Iraq.

* Corresponding author e-mail: sajad.abdulhasan@s.uokerbala.edu.iq https://doi.org/ 10.59658/jkas.v12i3.4349

Received:

Apr. 25, 2025

Accepted:

July 18, 2025

Published:

Sep. 15, 2025

Abstract

An experiment was carried out in the horticultural development project of the Directorate of Agriculture in Karbala, located in the Um Gharagher area / Al-Husseiniya District - Karbala Governorate, during the growing season 2024 to investigate the response of palm trees of the Barhi variety to fertilization with humic acid and bio-fertilizers in the chemical qualities of date palm leaves Barhi variety. The study was carried out as a factorial experiment (4×4) according to the Completely randomized block design (R.C.B.D). with three replicates, the study included two factors, the first is the addition of humic acid in four concentrations (0, 5, 10 and 15) g 20 liters per palm-1 in four batches and the second is the addition of bio-fertilizers in four concentrations (Control and adding Azotobacter chrooccum bacteria at a concentration of 150 ml 20 liters per palm-1 and Bacillus megatherium at a concentration of 150 ml 20 liters per palm -1 and Azotobacter chrooccum bacteria at a concentration of 75 ml + adding Bacillus megatherium bacteria at a concentration of 75 ml) 20 liters per palm -1. The results showed that the ground addition treatment of humic acid (A15) outperformed the rest of the coefficients by giving the highest averages in the total leaf content of chlorophyll, carbohydrates, protein, nitrogen, phosphorus, and potassium (0.970 mg g-1, 12.376%, 6.397%, 1.024%, 0.495% and 1.293%), respectively. The ground addition treatment of bio-fertilizers (B3) also gave the highest averages in the total leaves content of chlorophyll, carbohydrates, protein, nitrogen, phosphorus, and potassium (0.993 mg g-1, 12.535%, 6.397%, 1.047%, 0.503% and 1.290%), respectively. As for the bilateral interaction between the two study factors, the treatment (A15B3) excelled by recording the highest average of all the studied traits.

Keywords: *Phoenix dactylifera* L., Humic Acid, Biofertilizers, Qualitative traits.

Introduction

The date palm (*Phoenix dactylifera* L.) belongs to the palm family Arecaceae, and is considered an important and blessed fruit tree, and played an important role in the



Mesopotamian civilization as it is of great economic importance [1]. The original homeland of the date palm is Iraq, as it is one of the most important countries producing dates in the world, with the number of fruitful palm trees in Iraq reaching 11,242,749 palm trees with an average production of 735,353 tons per year [2]. The Barhi date palm variety is one of the most important commercial Iraqi varieties, as its fruits are characterized by their absence of the substance (Tannins) are astringent, and its fruits are eaten with khalal, tamr, and rutab [3].

One of the reasons for the low productivity of palm trees in the Arab countries in general and Iraq in particular is the lack of efficient use of available agricultural resources for palm production, reliance on traditional methods in service operations, and the non-application of modern technologies, including the fertilization process, as it is considered one of the factors that affect the growth and production of palm trees [4]. Organic and biological fertilization has a significant role in promoting tree growth and improving the quality of fruits and the amount of production of the tree, as organic and biological fertilizers contribute to the provision of mineral elements (N.P.K.) in a ready-made and straightforward manner by activating microorganisms in the soil [5]. [6] confirmed in a study he carried out on palm trees of Khastawy variety that the ground addition of humic acid at a concentration of (100) g tree-1 led to an increase in the content of leaves of total chlorophyll and (N.P.K.) compared to the comparison treatment. [7] found that the addition of humic acid at a concentration of (30) ml-1 to the date palm of Khastawy variety led to an increase in the chlorophyll content of leaves amounting to (35.24 and 40.40) mg 100 g-1 wet weight and its carbohydrate content and (N.P.K) amounted to (16.52, 18.38%, 0.989, 1.253%, 0.135, 0.207%, 1.053 and 1.097%) respectively for the two seasons compared to the Control treatment that gave the lowest average for the aforementioned characteristics.

[8] added Azotobacter chrooccum and Bacillus megatherium to Manzanillo olive trees increased the chlorophyll content in leaves and (NK) compared to the non-addition treatment. [9] indicated in an experiment carried out on olive seedlings of Qaisi variety to find out the effect of adding two types of bacteria, Azotobacter chroococcum with three concentrations (0, 30 and 60) ml plant-1 and Bacillus megatherium with three concentrations (0, 30 and 60) ml plant-1 on the growth of these seedlings, that the bilateral interaction between the two species, which was at a concentration of 6 ml plant-1, led to an increase in the carbohydrate content of leaves compared to the comparison treatment. Due to the occurrence of environmental pollution in the soil, water, and atmosphere as a result of the excessive use of chemical fertilizers, which is reflected in the growth of trees, the study aimed to know the effect of humic acid and bio-fertilizers in improving the chemical properties of the leaves of date trees, Barhi variety.

Materials and Methods

The experiment was carried out in the horticulture development project / Directorate of Agriculture of Holy Karbala, located in the Um Gharagher area of the



Husseiniya district / the holy province of Karbala during the period from 1/3/2024 to 1/11/2024. Forty-eight palm trees of the Barhi variety were selected at the age of 16 years, homogeneous in vegetative growth and similar in size and shape, planted with lines (8×8) m, irrigated by the basin method. The manual pollination of trees was carried out on 24/3/2024 using pollen of the red sheep variety, and the thinning process was also carried out on the clusters, leaving 8 clusters for each palm tree. The clusters were also concentrated and drooped.

Factors and design of the study: The first factor was the addition of humic acid in four concentrations (0, 5, 10 and 15) g 20 liters per palm-1 in four batches, the first one week before the Pollination process, the second batch in the Hababuk stage, the third batch in the Chamri stage, and the fourth batch in the khalal stage, which was symbolized (A0, A5, A10, A15). The second factor included the addition of bio-fertilizers in four concentrations (Control and adding *Azotobacter chroococcum* at a concentration of 150 ml 20 liters per palm-1, adding *Bacillus megatherium* at a concentration of 75 ml + adding *Bacillus megatherium* at a concentration of 75 ml) 20 liters per palm-1, and its symbol (B0, B1, B2, B3). The experiment was carried out according to a completely randomized block design (R.C.B.D), with three replications. Each replication included 16 palm trees, and with one palm tree per experimental unit, the number reached 48 palm trees.

Studied Traits:

Chlorophyll content (mg g-1 soft weight). The total chlorophyll content of soft leaves was estimated according to the method described previously[10].

Percentage of carbohydrates in leaves (%): Total carbohydrate Percentage was estimated following the method mentioned previously [11].

Percentage of total protein of leaves (%): The total protein percentage was estimated according to the method mentioned by [12] by applying the following equation: Percentage of protein in leaves = percentage of nitrogen in leaves $\times 6.25$

Concentration of mineral elements in the leaves: The leaf samples were dried in an electric oven at 70°C until the weight was constant. The dry samples were finely ground. The method [13] was followed in digesting the ground samples. The concentration of mineral elements was estimated:

- **1-Nitrogen concentration (%):** Total nitrogen in plant samples was estimated by the steam distillation (Kjeldahl) based on the method of [14].
- **2-Phosphorus concentration (%):** Estimated by a spectrophotometer at a wavelength of 400 nm after adjusting the acidity of the mixture using ammonium molybdate and ascorbic acid according to the method mentioned previously [15].
- **3-Potassium concentration** (%): was estimated using a flame photometer according to the method described previously [13].



Statistical analysis

The results of the study were analyzed statistically using the ANOVA Table according to the statistical program Genstat (2007), and the averages were compared according to the test of the Least Significant Difference (L.S.D) at a level of probability 0.05 [16].

Results and Discussion

The results shown in Table (1, 2, 3, 4, 5, and 6) showed that the ground addition treatment of humic acid (A15) significantly exceeded in all the studied traits by giving it the highest average in (total chlorophyll content, carbohydrate percentage, protein percentage, nitrogen, phosphorus and potassium concentration) amounting to (0.970 mg g⁻¹, 12.376%, 6.397%, 1.024%, 0.495% and 1.293%) respectively compared to the control treatment (A0), which gave the lowest average of (0.766 mg g⁻¹, 10.494%, 5.449%, 0.872%, 0.387% and 1.016%) respectively. It also excelled in The treatment of ground addition to bio-fertilizers (B3) significantly in all studied traits by recording the highest average in (total chlorophyll content, carbohydrate percentage, protein percentage, nitrogen, phosphorus and potassium concentration) amounted to (0.993 mg gram-1, 12.535%, 6.397%, 1.047%, 0.503% and 1.290%), respectively. Compared to the Control treatment (B0), which gave the lowest average of (0.765) mg g⁻¹, 10.565%, 5.449%, 0.863%, 0.400% and 1.055 %, respectively.

As for the interaction between the two factors of the study, the results showed that Binary interference treatment (A15B3) was significantly superior in all the studied traits by giving it the highest average in (total chlorophyll content, carbohydrate percentage, protein ratio, nitrogen, phosphorus and potassium concentration) amounting to (1.108 mg g⁻¹, 13.411%, 7.535%, 1.206%, 0.566% and 1.426%) respectively compared to the comparison treatment (A0B0), which gave the lowest average of (0.735 mg g⁻¹, 9.879%, 5.083%, 0.813%, 0.359% and 0.946%) respectively.

Table (1): Effect of Ground Addition of Humic Acid and Bio-fertilizers and Interaction between Them on Total Chlorophyll Content (mg⁻¹) in Date Palm Trees of Barhi Variety.

Humic acid	Bio-fert	Average			
20 g per Palm ⁻¹	В0	B1	B2	В3	humic acid
A 0	0.735	0.739	0.741	0.849	0.766
A 5	0.738	0.749	0.751	0.974	0.803
A 10	0.747	0.838	0.841	1.041	0.867
A 15	0.842	0.963	0.964	1.108	0.970



Journal of Kerbala for Agricultural Sciences Issue (3), Volume (12), (2025)

Average fertilizer	0.765	0.822	0.824	0.993	
I C D 005	Humic acid		Bio-fertilizers		Overlap
L. S .D 0.05	0.002		0.002		0.005

Table (2): Effect of Ground Addition of Humic Acid and Bio-fertilizers and the Interaction between Them on the Percentage of Carbohydrates in Leaves for Palm Trees of Barhi Variety %

Humic acid	Bio-fert	Average			
20 g per Palm ⁻¹	В0	B1	B2	В3	hu- mic acid
A 0	9.879	10.202	10.205	11.691	10.494
A 5	10.198	10.801	10.803	12.007	10.953
A 10	10.704	11.552	11.554	13.031	11.711
A 15	11.479	12.307	12.309	13.411	12.376
Average fertilizer	10.565	11.216	11.218	12.535	
L. S. D 0.05	Humic acid		Bio-fertilizers		Overlap
	0.0	011	0.011		0.023

Table (3): Effect of ground addition of humic acid and bio-fertilizers and the interaction between them on the percentage of protein in leaves for palm trees of the Barhi variety

Humic acid	Bio-fer	Average			
20 g per Palm ⁻¹	В0	B1	B2	В3	humic acid
A0	5.083	5.377	5.381	5.956	5.449
A5	5.354	5.456	5.458	6.204	5.618
A10	5.435	5.737	5.748	6.479	5.850
A15	5.697	6.172	6.183	7.535	6.397
Average fertilizer	5.449	5.618	5.850	6.397	
L. S. D 0.05	Humi	c acid	Bio-fertilizers		Overlap
	0.0	12	0.012		0.023



Table (4): Effect of ground addition of humic acid and bio-fertilizers and the interaction between them on the concentration of nitrogen in the leaves of palm trees of the Barhi variety

Humic acid	Bio-fer	Average			
20 g per Palm ⁻¹	B 0	B1	B2	В3	humic acid
A 0	0.813	0.860	0.861	0.953	0.872
A 5	0.857	0.873	0.873	0.993	0.899
A 10	0.870	0.918	0.920	1.037	0.936
A 15	0.912	0.988	0.989	1.206	1.024
Average fertilizer	0.863	0.910	0.911	1.047	
I C D 0.05	Humic acid		Bio-fertilizers		Overlap
L. S. D 0.05	0.002		0.002		0.004

Table (5): The effect of ground addition of humic acid and bio-fertilizers and the interaction between them on the concentration of phosphorus in the leaves of palm trees of the Barhi variety %

Humic acid	Bio-fertili	Average			
20 g per Palm ⁻¹	В0	B1	B2	В3	humic acid
A0	0.359	0.381	0.384	0.422	0.387
A5	0.377	0.415	0.417	0.481	0.423
A10	0.412	0.457	0.462	0.542	0.468
A15	0.451	0.480	0.483	0.566	0.495
Average fertilizer	0.400	0.433	0.437	0.503	
L. S. D 0.05	Humic	acid	Bio-fertilize		Overlap
	0.00)2	0.002		0.005



Table (6): Effect of ground addition of humic acid and bio-fertilizers and the interaction between them on potassium concentration in leaves for palm trees of Al-Barhi variety

Humic acid	Bio-fertili	Average			
20 g per Palm ⁻¹	B0 B1		B2	В3	humic acid
A0	0.946	0.977	0.983	1.156	1.016
A5	0.974	1.113	1.121	1.268	1.119
A10	1.113	1.187	1.193	1.309	1.201
A15	1.186	1.278	1.284	1.426	1.293
Average fertilizer	1.055	1.139	1.145	1.290	
L.S. D 0.05	Humic	Humic acid		tilizers	Overlap
	0.002		0.002		0.004

The results show that the addition of humic acid has a positive effect in increasing all the studied traits, and the reason for this increase may be attributed to the role of humic acid in reducing soil pH and increasing the permeability of the cell membranes of the root system, which leads to facilitating the transfer of nutrients to the plant [17]. Alternatively, perhaps the reason is due to the role of nitrogen-containing fertilizer in the construction of chlorophyll pigment and the formation of porphyrin groups important in the synthesis of this dye [18], which reflected positively on the increase in the total chlorophyll content in the leaves. As for the reason for the increase in the percentage of carbohydrates, it may be due to its role of humic acid in increasing the chlorophyll content in the leaves Table (1) and improving the process of absorption of water and nutrients necessary for the plant due to its ability to increase the permeability of cell membranes, which leads to an increase in the efficiency of the carbon metabolism process in the leaves and thus increase the accumulation of carbohydrates in the leaves [19, 20].

As for the increase in the concentration of mineral elements (nitrogen, phosphorus and potassium) in the leaves Table (4, 5 and 6) respectively, the reason for this may be attributed to the role of acid, which is a storehouse of nutrient mineral elements, as it improves ketone exchange and increases the readiness of mineral elements, which facilitates the process of absorption of nutrients by the plant due to its important and direct role in the permeability of the cell membranes of roots and leaves and thus increasing the amount of these elements within the plant [21]. As for the increase in the protein percentage in the leaves Table (3), the reason may be attributed to its role in increasing the nitrogen element in the leaves Table (4), as nitrogen contributes to



increasing the representation of some of the products of photosynthesis into protein through a series of enzymatic reactions that in turn convert ketone organic acids (Oxaloacetis acid and a-Ketoglutaric acid) into amino acids, which in turn are linked to each other by peptide bonds, resulting in protein formation [22]. These results are consistent with what was found by [23, 6,7] in their studies on date palm (Al-Khastawi) varieties.

As for the explanation of the positive effect of bio-fertilizers in increasing the content of chlorophyll in the leaves Table (1), the reason for this may be due to the role of biofertilizers in increasing the readiness of the necessary and important elements for growth in the plant, such as nitrogen and phosphorus, in addition to their efficiency in reducing the soil pH, which leads to increasing the readiness of some of the microelements which the plant needs mainly [24]. Also, the increased carbohydrates percentage, protein percentage and concentration of mineral elements (N.P.K.) in the leaves Table (2,3,4, 5 and 6) respectively, may be due to the addition of bio-fertilizers in increasing the concentration and readiness of the mineral elements N.P.K. in the soil solution, which was positively reflected in increased their absorption by the root system and increasing the transfer of these elements to the leaves. This is confirmed by a number of researchers that increasing the concentration of elements in the soil leads to increased absorption by plants [25]. In addition, the ability of Azotobacter chrooccum and Bacilius megatherium bacteria to dissolve organic phosphorus, which increases the absorption process in the roots, which is reflected in the increased absorption of nutrients from the soil solution, which leads to an increase in the food processing process and an increase in the proportion of carbohydrates, and by increasing vegetative and root growth, the need for nitrogen absorption by the plant increases, increasing its accumulation in the form of proteins and amino acids [26 and 27]. These results are consistent with the findings of [28] in his study on palm trees of the Zahdi and Ashrasi varieties. The reason for the increase in all the characteristics mentioned as a result of the bilateral interference between the two study factors recorded by the binary interference factor (A15B3) may be attributed to the aggregate effect between the two study factors.

Based on the above results, we conclude that the addition of humic acid (A15) and the ground addition of bio-fertilizers (B3) led to an improvement in the chemical content of palm leaves of the Barhi variety.

References

- 1) Ibrahim, A. B. O. (2014). *Date palm: History, heritage, food and medicine*. Isa Cultural Center. (327 pp.).
- 2) Central Bureau of Statistics. (2020). *Annual statistical collection*. Ministry of Planning, Department of Publishing and Public Relations.



- 3) Al-Hamdani, K. A. S., Mohammed, M. H., & Hussein, M. N. (2022). *Date palm* (1st ed.). Tikrit University Press. (340 pp.).
- 4) Al-Tamimi, I. H. (2012). The effect of adding parallel proportions of chemical fertilizers on the growth of date palm shoots (Phoenix dactylifera L., Barhi variety). *Basra Journal of Date Palm Research*, 38(4).
- 5) Jassim, A. R. A. L., Al-Dahan, M. R. A., & Abboud, S. M. (2017). *Date palm tree service technologies*. University House for Printing, Publishing and Translation, Ministry of Higher Education and Scientific Research.
- 6) Al-Aqabi, A. H. K. (2022). Effect of ground addition of humic acid and spraying with marine algae extract on some growth characteristics and yield of green dates variety [Master's thesis, Diyala University, College of Agriculture].
- 7) Kredi, E. H. M., & Al-Ali, H. H. (2023, April). Response of date palm trees (Phoenix dactylifera L., Khastawi cultivar) to chemical, organic and biological fertilization. *IOP Conference Series: Earth and Environmental Science*, 1158(4), 042011.
- 8) Hassan, H. S. A., Abd-Alhamid, N., Haggag, L. F., & Hassan, A. M. (2015). Effect of organic and bio-fertilization on vegetative growth and leaf mineral contents of Manzanillo olive trees. *Middle East Journal of Agricultural Research*, 4(4), 899–906.
- 9) Hamza, S. Q. (2021). The effect of adding bio-fertilizers on growth, mineral and medicinal content of olive seedling leaves, Qaisi variety [Master's thesis, University of Thi-Qar, College of Agriculture and Marshes].
- **10)** Mahadevan, A., & Sridhar, R. (1986). *Methods in physiological plant pathology* (p. 316). Sivakami Publications.
- 11) Joslyn, M. A. (1970). Methods in food analysis: Physical, chemical, and instrumental methods of analysis (2nd ed.). Academic Press.
- 12) Al-Tamimi, I. B. H. (2006). Using mathematical modeling to predict date palm productivity (Phoenix dactylifera L., Dessert variety) under the influence of heterogeneity of some production factors [Doctoral dissertation, University of Basra, College of Agriculture].
- 13) Cresser, M. S., & Parsons, J. W. (1979). Sulphuric-perchloric acid digestion of plant material for the determination of nitrogen, phosphorus, potassium, calcium, and magnesium. *Analytica Chimica Acta*, 109(2), 431–436.
- **14)** Page, A. L., Miller, R. H., & Kenney, D. R. (1982). *Methods of soil analysis:* Part 2 (2nd ed.). Agronomy Monograph No. 9. American Society of Agronomy.
- **15)** Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36.



- **16)** Al-Mohammadi, S. M., & Al-Mohammadi, F. M. (2012). *Statistics and experiment design*. Dar Osama for Publishing and Distribution.
- 17) Muslat, M. M., & Musleh, O. H. (2015). *The basics of organic farming*. Sky Press, Faculty of Agriculture, Anbar University.
- **18)** Kandil, E. A., Fawzi, M. I. F., & Shahin, M. F. M. (2010). The effect of some slow release nitrogen fertilizers on growth, nutrient status, and fruiting of "Mit Ghamr" peach trees. *Journal of American Science*, 6(12), 195–201.
- 19) Khaled, H., & Hassan, A. F. (2011). Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil & Water Research*, 6(1), 21–29.
- **20)** Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction between macroand micro-nutrients in plants. *Frontiers in Plant Science*, *12*, 665583. https://doi.org/10.3389/fpls.2021.665583
- **21)** Van Oosten, M. J., Pepe, O., De Pascale, S., Silletti, S., & Moggio, A. (2017). The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. *Chemical and Biological Technologies in Agriculture*, 4(1), 5.
- **22)** Aczel, M. R. (2019). What is the nitrogen cycle and why is it key to life? *Frontiers for Young Minds*, 7(41), Article 9. https://doi.org/10.3389/frym.2019.00041
- 23) Al-Hamdani, K. A. S. (2016). Effect of humic acid and K-humate treatment on vegetative growth, quantitative qualities of fruits, and nutrient content of date palm trees of Khastawy variety growing in gypsum soils. *Diyala Journal of Agricultural Sciences*, 8(1), 218–231.
- **24)** Raimi, A., Roopnarain, A., Chirima, G. J., & Adeleke, R. (2020). Insights into the microbial composition and potential efficiency of selected commercial bio-fertilisers. *MicrobiologyOpen*, 6(7), e04342. https://doi.org/10.1002/mbo3.4342
- **25)** Mosa, W. F. A., Paszt, L. S., Frąc, M., Trzciński, P., Treder, W., & Klamkowski, K. (2018). The role of biofertilizers in improving vegetative growth, yield, and fruit quality of apple. *Horticultural Science (Prague)*, 45(4), 173–180.
- **26)** Nosrati, R., Owlia, P., Saderi, H., Rasooli, I., & Malboobi, M. A. (2014). Phosphate solubilization characteristics of efficient nitrogen-fixing soil *Azotobacter* strains. *Iranian Journal of Microbiology*, *6*, 285–295.
- 27) Hafez, M., Albarbarary, T. A., Ibrahim, I., & Abdel-Fatah, Y. (2016). *Azoto-bacter vinelandii*: Evaluation and optimization of Abu Tartur Egyptian phosphate ore dissolution. *Saudi Journal of Pathology and Microbiology*, 1(3), 80–93.
- **28)** Homad, Ahmed Thamer (2021). Effect of Biofertilizer Use and Coverage on Chemical Content and Yield of Ascetic and Ashrasi Palm Varieties. PhD thesis, Faculty of Agriculture. Diyala University. Iraq.