Effect of Humic and Fulvic Acids Application on growth and Flowering of *Catharanthus roseus* L.

Hassan Hadi Mustfa[1]*, Abdul Kareem A.J. Mohammad Saeed [2*] ,and Eman Hekmet Hassan
[1]Department of Soil Sciences and Water Resources ,College of Agriculture, University of Diyala ,Iraq,
[2]Department of Horticulture and Landscape ,College of Agriculture, University of Diyala ,Iraq.
[3]Department of Horticulture and Landscape ,College of Agriculture, University of Diyala ,Iraq.
*Corresponding authors email : emanhekmet@ uodiyala.edu.iq
hassanalalawy@uodiyala.edu.iq [*],

Abstract
An experiment was conducted during the season 2022-2023 in the greenhouse to study the impact of adding humic acid at concentrations of 0, 50 and 100 kg ha^{-1} as ground addition and fulvic acid at concentration of 0, 5 and 10 mg L^{-1} as foliar spraying on vegetative and flowering growth traits *Catharanthus roseus* L.,. The experiment was designed as a factorial experiment (3×3) in Randomized Complete Blocks Design (RCBD) with three replicates.
The results showed that adding humic acid as ground addition at concentration of 100 kg ha^{-1} was superior on increasing plant height (58.00 cm), number of branches (5.33), number of leaves (185.50) , leaf area (19.50 cm^2), relative chlorophyll content in the leaves (52.0 SPAD), stem diameter (1.80 cm) and number of the flowers (5.00). Application of fulvic acid as foliar spraying improved all vegetative and flowering traits. Treatment of 10 g L^{-1} was the most significant effect in increasing plant height (49.16), number of branches (4.50), number of leaves (152.66), leaf area (18.51 cm^2), relative chlorophyll content (51.11 SPAD), Stem diameter (1.50 cm) and number of flowers (4.00). The interaction effect between the studied factors was significant in all studied traits. Humic acid treatment at concentration of 100 kg ha^{-1} with fulvic acid at concentration of 10 mg L^{-1} was the most effective and gave the best results in terms of plant height (61.50), number of branches (6.50), number of leaves (210.00), leaf area (20.60 cm^2), relative chlorophyll content (55.35 SPAD), Stem diameter (1.95 cm) and number of flowers (5.50).

Keywords: Humic acid, Fulvic acid, *Catharanthus roseus*, vegetative growth, flowering growth.

Introduction
as periwinkle in Madagascar, it is an indigenous species of the genus Catharanthus (14). Ornamental plant cultivation thrives in soils rich in organic matter with good drainage and ventilation. Humic compounds are natural organic compounds obtained from decomposing plants and organic fertilizer. Humic substances are of great importance in plant nutrition because they work to increase the

Catharanthus roseus L. belongs to the Apocynaceae family. It is an ornamental plant whose cultivation is widespread in all tropical, subtropical and warm temperate regions. It is considered one of the perennial plants that can be renewed annually and grows to a height of 30-60 cm (9, 10). There are two common types of Vinca, named for the color of their flowers, pink 'Roseau' and white 'Alba'. Known

ISSN 2072-3857

IAA oxidase enzyme, thus increasing the activity of the Indole acetic acid hormone (4) fulvic acid is one of the humic components and is formed in the early stages of humus formation. It has a yellow or yellowish-brown color and is dissolved in water under acidic and basic conditions. It has a low molecular weight (11). It is the second most important humus substance and one of the main bio stimulants that work to improve plant production (5), it also attracts water molecules and facilitates the movement of trace elements in the roots and acts as a chelating substance for metals (15). Given the importance of nourishing and improving plant growth using fertilizers from low-cost natural sources and without harming the environment, the study aimed to demonstrate the effect of both addition, ground of

ratio of 1:3 table No. (1). The study included two factors, the first was adding humic acid as ground addition at concentrations of 0, 50 and 100 kg ha^{-1}, and the second was fulvic acid as foliar spraying at concentration of 0, 5 and 10 g L^{-1}. The study was carried out as a factorial experiment (3×3) according to a completely randomized block design (RCBD), with three replicates and five plants per experimental unit. The data were analyzed according to the statistical program (SAS, 2002). Means were compared using Duncan multiple tests at a probability of 0.05. The experimental measures included the plant height (cm), leaf area (cm^2), number of leaves, number of branches, relative chlorophyll content in leaves (SPAD), stem diameter (cm), and number of Flowers.

release of nutrients and increase the cation exchange capacity of ions. It increases the availability of micronutrients, is an environmentally friendly compound, improves the chemical and physical properties of the soil, and is low in cost compared to chemical fertilizers. Humic acid is considered a commercial product that is widely used because it contains many important nutritional elements necessary for plant growth (2, 7) Humic acid is produced naturally from humic compounds resulting from the decomposition of organic matter, and it works to increase the absorption of nutrients by the plant, especially in periods of drought. It increases the proteins in the plant and the numbers of beneficial microorganisms in the soil (8, 1), and increases it brings together sugars, enzymes, and amino acids and develops chlorophyll (6). It also works to inhibit the activity of the humic acid and spraying with fulvic acid on the vegetative and flowering growth of periwinkle plant (*Catharanthus roseus* L.).

Materials and methods

The study was carried out in one of the greenhouses affiliated with the research station of the Soil Sciences and Water Resources Department / College of Agriculture / University of Diyala, for the fall season 2022-2032,from 1/11/2022 to 1/6/2023 to study the effect of humic acid as ground addition and fulvic acid as foliar spraying on growth and flowering of *Catharanthus roseus*. Seedlings of the plant were brought from one of the private nurseries in Baqubah district and were approximately one month old and transferred to a pots filled with soil consisting of loam + peat moss in a

ISSN 2072-3857

Table No. (1) Some chemical and physical properties of agricultural soil

Measures class	Measuring unit	The value
electrical conductivity (EC) 1:1	ds m^{-1}	0.6
P^H 1:1	……..	7.34
available nitrogen N	kg^{-1} soil	25
available phosphorous P		1.18
available potassium K		256.12
organic matter	G kg^{-1}	7.9
calcium carbonate $CaCO_3$		261.00
dissolved calcium Ca^{+2}	Mill equivalent liter	7.8
dissolved magnesium Mg^{+2}		34
dissolved sodium Na^{+2}		4.43
dissolved bicarbonate HCO_3		1
dissolved chlorine Cl		16.5
dissolved potassium K		1.02

Results

1- Plant height (cm)

plant height (30.66 cm). Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} also excelled by recording the highest plant height (49.16 cm) compared to the control treatment, which recorded the lowest plant height (40.00 cm).

Table 1 shows that there are significant differences between the treatments, as the humic acid addition treatment excelled and recorded the highest plant height at concentration of 100 kg ha^{-1} (58.00 cm) compared to the control treatment, which recorded the lowest

ISSN 2072-3857

Table 2: Impact of humic and fulvic acids application on plant height (cm) of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L $^{-1}$)			Average
	0	5	10	
0	00.20 f	35.00 e	37.00 e	30.66 C
50	44.50 d	47.00 cd	49.00 c	46.83 B
100	55.50 b	57.00 b	61.50 a	58.00 A
Average	40.00 C	46.00 B	49.16 A	

branches (2.50 branches plant^{-1}). Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} also excelled by recording the highest number of branches per plant (4.50 branches plant^{-1}) compared to the control treatment and spraying treatment at concentration 5 g L^{-1}, which recorded 3.16 and 3.66 branches per plant respectively. Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 6.50 branches per plant compared to the control treatment which recorded the lowest number of branches per plant (3.00).

Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 61.50 cm compared to the control treatment which recorded the lowest plant height (20.00 cm).

Number of branches (branches plant^{-1})

Table 2 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest number of branches (5.33 branches plant^{-1}) at concentration of 100 kg.ha^{-1} compared to the control treatment, which recorded the lowest number of

ISSN 2072-3857

Table 3: Impact of humic and fulvic acids application on number of branches (branches plant^{-1}) of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	2.00 e	2.50 de	3.00 cde	2.50 C
50	3.00 cde	3.50 bcde	4.00 bcd	3.50 B
100	4.50 bc	5.00 ab	6.50 a	5.33 A
Average of Fulvic acid	3.16 B	3.66 B	4.50 A	

compared to the control treatment, which recorded the lowest number of leaves per plant (120.66). Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 210.00 leaves plant^{-1} compared with the control treatment which recorded the lowest number of leaves per plant (94.00).

3-Number of leaves (leaf plant $^{-1}$)

Table 3 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest number of leaves per plant (185.50) at the concentration of 100 kg ha^{-1} compared to the control treatment, which recorded the lowest number of leaves (88.83). Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} also excelled by recording the highest number of leaves per plant (152,660)

Table 4: Effect of adding humic acid and spraying with fulvic acid on the number of leaves plant^{-1} of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	82.00 f	90.50 f	94.00 f	88.83 C
50	112.00 e	137.50 d	154.00 c	134.66 B
100	167.50 b	179.00 b	210.00 a	185.50 A
Average of Fulvic acid	120.66 B	135.66 B	152.66 A	

ISSN 2072-3857

4- Leaf area (cm^2)

which recorded the lowest leaf area (17.10 cm^2). Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 20.0 cm^2 compared with the control treatment which recorded the lowest leaf area (16.50 cm^2).

Table 4 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest leaf area (19.50 cm^2) at concentration of 100 kg ha^{-1} compared to the control treatment, which recorded the lowest leaf area (15.86 cm^2). Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} also excelled by recording the highest number of leaf area (18.51 cm^2) compared to the control treatment,

Table 5: Impact of humic and fulvic acids application on leaf area (cm2) of Catharanthus roseus L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	15.25 f	15.85 ef	16.50 de	15.86 C
50	17.00 d	17.90 c	18.45 bc	17.78 B
100	18.80 b	19.10 b	20.60 a	19.50 A
Average of Fulvic acid	17.10 C	17.61 B	18.51 A	

(Relative chlorophyll content in leaves (SPAD

ha-1 compared to the control treatment and humic acid at concentration of 50 kg ha-1, which recorded the lowest relative chlorophyll content in leaves which recorded 44.00 and 47.71 .SPAD, respectively

Table 5 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest relative chlorophyll content in leaves (52.60 SPAD) at the concentration of 100 kg

ISSN 2072-3857

Table 6: Impact of humic and fulvic acids application on relative chlorophyll content in leaves (SPAD) of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	40.00 d	43.60 cd	47.65 bcd	44.00 B
50	45.40 cd	47.40 bcd	50.35 abc	47.71 B
100	52.90 b a	49.55 abc	55.35 a	52.60 A
Average of Fulvic acid	46.35 B	46.85 B	51.11 A	

interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 55.35 SPAD compared with the control treatment which recorded the lowest relative chlorophyll content in leaves (47.65 SPAD).

Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amounting to 1.95 cm however; it was on par with interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 5 g L^{-1}) which recorded 1.80 cm compared with control treatment which recorded the lowest stem diameter (1.15 cm).

Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} also excelled by recording the highest relative chlorophyll content in leaves (51.11 SPAD) compared to the control treatment and fulvic acid foliar spraying treatment at concentration of 5 g L^{-1}, which recorded the lowest relative chlorophyll content in leaves which recorded 46.35 and 46.85 SPAD, respectively. Regarding the

Stem diameter (cm)

Table 6 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest stem diameter (1.80 cm) at concentration of 100 kg ha^{-1} compared to the control treatment, which recorded the lowest stem diameter (0.98 cm). Fulvic acid foliar spraying treatment of 10 g L^{-1} gave the best results in term of stem diameter (1.50 cm) however; it was on par with fulvic acid treatment of 5 g L^{-1} which recorded 1.43 cm compared to the control treatment, which recorded the lowest stem diameter (1.25 cm).

ISSN 2072-3857

Table 7: Impact of humic and fulvic acids application on stem diameter (cm) of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	0.75 e	1.05 de	1.15 cd	0.98 C
50	1.35 bcd	1.45 bc	1.40 bc	1.40 B
100	1.65 ab	1.80 a	1.95 a	1.80 A
Average of Fulvic acid	1.25 B	1.43 A	1.50 A	

compared to the control treatment, which recorded the lowest number of flowers, amounting to 2.83 flowers plant^{-1}. Regarding the interaction between the studied treatments, we note the superiority of the interaction treatment (humic acid as ground addition at concentration of 100 kg ha^{-1} with fulvic acid as foliar spraying at concentration of 10 g L^{-1}) amount of 5.50 flowers per plant^{-1} compared with control treatment which recorded the lowest number of flowers per plant (2.55).

Number of Flowers (flower plant^{-1})

Table 7 shows that there are significant differences between the treatments, as the humic addition treatment excelled and recorded the highest number of flowers per plant (5.00) at the concentration of 100 kg ha^{-1} compared to the control treatment, which recorded the lowest number of flowers per plant (2.00), amounting to 2.00. Fulvic acid foliar spraying treatment at concentration of 10 g L^{-1} excelled by recording the highest number of flowers, reaching 4.00 flowers plant^{-1}

Table 8: Impact of humic and fulvic acids application on number of flowers (flower plant^{-1}) of *Catharanthus roseus* L.

Humic acid (kg ha^{-1})	Fulvic acid (g L^{-1})			Average of Humic acid
	0	5	10	
0	1.50 e	2.00 de	2.50 cde	2.00 C
50	2.50 cde	3.50 bcd	4.00 abc	3.33 B
100	4.50 ab	5.00 ab	5.50 a	5.00 A
Average of Fulvic acid	2.38 B	3.50 AB	4.00 A	

ISSN 2072-3857

Discussion

acid contains a group of quinine, this group acts as a hydrogen receptor and thus increases the activity of enzymes, in addition to its role in the processes of photosynthesis and respiration. It also has a role in interacting with phospholipid compounds present in cell membranes. These compounds work as a carrier for transporting nutrients from the outside to the inside of the cell, it therefore increases the permeability of the cellular membranes and increases the absorption of water and nutrients such as nitrogen and iron, as 70% of the nitrogen present in the leaf along with magnesium enters into the formation of the chlorophyll pigment, and 80% of the iron element is present in the chloroplasts and is thus reflected. This improves the process of photosynthesis and building plastids (13., 12).

The results showed that the ground addition of humic acid and spraying with fulvic acid had significant effects on most of the traits under study, as it was noted that the superiority of humic acid as ground addition at the concentration of 100 kg ha^{-1} and fulvic acid as foliar spraying at concentration of 10 g L^{-1} may be attributed to the importance role of humic acid in plant nutrition. Humic and fulvic acids increases chlorophyll content because these acids contain a group of nutritional elements such as nitrogen, phosphorus, potassium, and some micronutrients, which participate in the synthesis of chlorophylls, which are important in the process of photosynthesis (3), or it may be due to the role of humus (fulvic and humic acids) in increasing the various biological and physiological activities necessary for plant growth, as humic

References

1-Al-Allaf, Iyad Hani Ismail. 2012. The effect of adding urea and humic acid on the growth of Alenki seedlings. Mesopotamian Agriculture Journal, 40 (4): 22-31.

2-Al-Aty, Wissam Naji Ahmed. 2015. The effect of the moisture preservative Driwater and humic acid on the physiological characteristics and active substances of the chrysanthemum plant. College of Agriculture, Tikrit University.

3-Al-Tamimi, Muhammad Salal Aliwi and Al-Jadiri, Dhay Muhammad Matar. 2017. The effect of adding poultry waste and spraying with organic acids on some growth characteristics and yield of potatoes. Al-Furat Journal of Agricultural Sciences/ Supplement No. 4 527-540.

4-Aml, R.M. Yousef, Hala. S. Emam and M.M. S.Saleh .2011. Olive seedling growth as affected by humic and amino acids macro and trace elements applications .Agriculture and Biology Journal of North America.(7): 1101-1107.

5-Capstaff, N.M. Morrison, F.Cheema, J. Brett, P. Hill, L. Munoz Garcia , J.C.K. Himyak, Y.Z.M. Domoney, C. Miller, A.J., 2020. Fulvic acid increases forage legume growth inducing preferential up regulation of nodulation and signaling – related genes. Journal of experimental botany, 71(18), 5689-5704.

6-Chen, Y.M. Nobili and T. Aviad. 2004. Stimulatory effect of humic substances on plant growth. In. Magdoft F. Ray R. (eds): soil Organic Matter in Sustainable Agriculture. CRCPress. Washington.

ISSN 2072-3857

7-EL-Tahlawy, Y.A. and Ali, O.A. 2022. Role of Humic Substances on growth and yield of crop plant. Biostimulants for crop production and sustainable Agriculture, 159.

8-Hartwigson, I.A and M.R. Evans .2000. Humic acid seed and substrate treatment promote seedling root development. Horticulture Science. 35(7): 1231-1233.

9-Jaleel, C.A.B. Sankar, R. Sridharan and R. Panneerrselvam. 2008. Soil salinity alters growth chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus.* Turk. Biol., (32):79-83.

10-Kessler, J.R. 1998. Greenhouse production of annul Vinca. Alabama Cooperative Extenion System, New July, ANR-1119.

11-Melendrez, M.M. 2020. Humic acid the science of humus and how it benefits soil. Eco Farming Daily. On line Available from: https:// www.ecofarmingdaily. Com /build-soil /humus /humic –acid.

12-Osman, S.M.A. Khamis and Thorya, 2010. Effect of mineral and Bio-NPK soil application on vegetative growth, flowering, fruiting and leaf chemical composition of young olive trees. Res. J. Agric. And Biol. Sci. 6(1):54-63.

13-Pinton, R., Z. Varanini and G. Vizzoto, 1992. Humic substances affect transport properties of tonoplast vesides isolated from oat root. Plant and soil. The Hange, V. 42:203-210.

14-Sain, M., and Sharma V., 2013. *Catharanthus roseus* (an anti-cancerous drug yielding plant) - a review of potential therapeutic properties. International Journal of Pure & Applied Bioscience, 1(6), 139-142.

15-Wang, S. Tian, X., and Liu Q., 2020. The effectiveness of foliar application of zinc and biostimulants to increase zinc concentration and bioavailability of wheat grain . Agronomy, 10(2) 178.

ISSN 2072-3857