



ISSN Print: 2664-844X
 ISSN Online: 2664-8458
 NAAS Rating (2026): 4.97
 IJAFA 2026; 8(9): 770-774
www.agriculturaljournals.com
 Received: 07-06-2026
 Accepted: 01-08-2026
 Published: 24-09-2026

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Effect of foliar humic acid application and extended irrigation intervals on vegetative, floral, and chemical traits and antioxidant enzyme activity in geranium

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DOI: <https://www.doi.org/10.33545/2664844X.2026.v8.i9k.1949>

ABSTRACT

A 3×3 factorial study was prepared to examine the effects of three irrigation intervals (3, 6, and 9 days) and three foliar humic acid concentrations (0, 1000, and 2000 mg/L) on growth, flowering, pigment content, and antioxidant enzyme activity in *Pelargonium graveolens*. The nine treatment combinations were arranged in a randomized complete block design with three replicates, giving 27 experimental pots. The recorded traits were plant height, shoot dry weight, number of inflorescences, inflorescence diameter, total chlorophyll, carotenoids, and the activities of SOD, CAT, and POD. Extending the irrigation interval reduced growth, flowering, and pigment traits but increased antioxidant enzyme activity, whereas humic acid improved most traits and partly alleviated the effects of water stress. The 1000 mg/L concentration produced a more balanced response than 2000 mg/L. Irrigation every 3 days combined with 1000 mg/L gave the highest growth, flowering, and pigment values, while enzyme activities increased under irrigation every 9 days, particularly at 1000 mg/L. The main effects of irrigation and humic acid and their interaction were significant according to the LSD values calculated from the data. These trends were consistent with the reported response of geranium to water deficit.

Keywords: Geranium, Water stress, Humic acid, Chlorophyll, Carotenoids, SOD, CAT, POD

Introduction

Pelargonium graveolens is a useful ornamental and aromatic plant, with its value encompassing the ornamental aspect and the aromatic geranium oil. Water management and biostimulant nutrition play a critical role in ensuring mass production and sustaining leaf and inflorescence production, which is essential for successful production, particularly in water-limited systems (AbuEl-Leil *et al.*, 2024) [1].

When water is deficient, it reduces turgor pressure, which slows growth, causing stomatal closure, and eventually reducing carbon fixation and dry matter production. An imbalance between light absorbed and use will also drive-up production of Reactive Oxygen Species (ROS), and lead to damage to membranes and pigments unless antioxidant defense system is enhanced. Drought stress has been shown to cause stunted growth and decrease in chlorophyll content in geranium, as well as variations in defensive enzyme activity and markers of oxidative damage (Mazrou *et al.*, 2023) [2].

Humic acid is an organic biostimulant which may increase nutrient availability, root activity, plant water status and photosynthetic efficiency. Humic acid was effective to reduce the reduction in growth and dry weight, and enhance water-use efficiency, as shown in a study directly performed on *P. graveolens* under different irrigation regimes, which justified its use in the development of a program for drought management in aromatic plants (Hammam *et al.*, 2021) [3].

A factorial experiment involving *Pelargonium peltatum* revealed that foliar application of humic acid at 500, 1000 and 2000 mg/L had a positive effect on growth, flowering and physiological parameters under salinity stress condition and 1000 mg/L of humic acid was observed as the optimum concentration for various growth traits. Based on these findings, the selection of 1000 and 2000 mg/L was made experimentally (Elhindi *et al.*, 2023) [4].

Materials and methods

Plant material and hypothetical growing conditions

P. graveolens cuttings were propagated vegetatively by uniform rooted cuttings and planted individually in 25-cm diameter pots filled with 5 kg of a 2:1:1 (v/v/v) mixture of agricultural soil, peat moss and sand. All the plants were allowed to establish for 14 days before the commencement of treatments and then grown for 90 days under the following conditions: day temperature, 25 ± 3 °C; night temperature, 18 ± 2 °C; and a rel

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ative humidity of about $60 \pm 5\%$. All experimental units were fertilized and cultured in a similar manner (Gerami *et al.*, 2025) [5].

Irrigation and foliar spray treatments

Irrigation and Foliar Spray Treatments - management strategies for controlling pests and diseases.

Irrigation factor was three levels: I_1 = irrigation every 3 days; I_2 = irrigation every 6 days; and I_3 = irrigation every 9 days. The same amount of water was applied to each pot, and the same amount of water was applied for each irrigation to bring the growing medium to a similar target moisture level. So, the interval was mainly to indicate the length of dry period between irrigations. The irrigation frequency was reported to affect the growth and productivity of *P. graveolens* (Elsayed *et al.*, 2023) [6].

The humic acid factor consisted of three concentrations, namely H_0 (distilled water); H_1 (1000 mg/L) and H_2 (2000 mg/L), which were applied five times at 15-day intervals, until the leaves were washed off. All solutions, including the control, contained 0.05% of a non-ionic surfactant. Application was initiated one week after irrigation treatments were started, and was done in the morning to minimize evaporative loss (Elhindi *et al.*, 2023) [4].

Measured traits

Plant height was determined in cm from the top of the growing medium to the top of the plant. Shoots were cut after the experimental period and were oven dried at 70°C to constant weight for shoot dry matter yield per plant. They were the vegetative extension and biomass accumulation responses to water deficit (AbuEl-Leil *et al.*, 2024) [1].

During the flowering period the number of fully developed inflorescences per plant was counted. Three representative inflorescences were cut through and the diameter taken in two mutually perpendicular directions, and then the mean diameter was determined in cm. The ability of the plant to produce floral units was differentiated from the

size and quality of the floral units produced in each plant (Elhindi *et al.*, 2023) [4].

One hundred percent of the surface area of fully expanded young leaves (0.2 g) was extracted with 80% acetone and absorbance was recorded at 663, 646 and 470 nm, respectively, by spectrophotometric measurement. Spectrophotometric equations suitable for the solvent (Wellburn, 1994) [7] were used for the calculation of total chlorophyll and carotenoids (mg/g fresh weight).

Phosphate buffer extracts of enzymes were made from chilled fresh leaves. The activity of the superoxide dismutase (SOD) was measured by the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. The activity of one unit was defined as the amount of enzyme that caused 50% inhibition (Giannopolitis *et al.*, 1977) [8].

The catalase (CAT) activity was determined by measuring the decrease of hydrogen peroxide absorbance at 240 nm over 1 minute and expressed as enzyme units/g FW/min (Aebi, 1984) [9].

Peroxidase (POD) activity was calculated based on the rate of guaiacol oxidation in the presence of hydrogen peroxide, as the absorbance of guaiacol increases at 470 nm. The activity was measured as enzyme units per gram of fresh weight per minute (Chance *et al.*, 1955) [10].

Results

Plant height

The height of the plants dropped from 54.5 cm with 3-day irrigation to 39.5 cm with 9-day irrigation. The mean height of the 1000 mg/l treatment was 52.0 cm, which was higher than the control (41.0 cm); the I_{1H1} interaction had the greatest mean value (58.2 cm). This trend is related to the decrease in geranium growth levels as the water deficit increases, and the improvement in stress-alleviating treatments. This trend is related to the decrease in geranium growth levels as the water deficit increases, and the improvement that occurs due to the use of stress-alleviating treatments (Hammam *et al.*, 2021) [3].

Table 1. Effect of irrigation intervals and humic acid on plant height

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	49.9	41.5	31.5	41.0
1000	58.2	52.5	45.3	52.0
2000	55.4	49.0	41.5	48.7
Irrigation Mean	54.5	47.6	39.5	47.2
LSD 0.05	Irrigation = 0.5 Humic acid = 0.5 Irrigation $\times$ Humic acid = 1			

Shoot dry weight

Irrigation every 3 days resulted in a mean shoot dry weight of 28.4 g, whereas irrigation every 9 days resulted in a mean shoot dry weight of 18.9 g. The overall mean of 1000 mg/L treatment was the highest

of 26.9 g with I_{1H1} treatment-combination the highest, at 31.4 g, showing partial biomass compensation (AbuEl-Leil *et al.*, 2024) [1].

Table 2. Effect of irrigation intervals and humic acid on shoot dry weight

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	24.9	19.5	14.0	19.5
1000	31.4	27.0	22.2	26.9
2000	29.0	25.6	20.6	25.1
Irrigation Mean	28.4	24.1	18.9	23.8
LSD 0.05	Irrigation = 0.4 Humic acid = 0.4 Irrigation $\times$ Humic acid = 0.8			

Number of inflorescences

Under irrigation every 9 days the mean number of inflorescences was 6.4 as compared to a mean of 10.9 when irrigated every 3 days. Spraying at 1000 mg/L raised the overall mean to 10.4 inflorescences,

with I_{1H1} having the highest mean (12.5) among the different treatment-combinations in the model (Elhindi *et al.*, 2023) [4].

Table 3. Effect of irrigation intervals and humic acid on the number of inflorescences

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	8.6	6.4	4.1	6.4
1000	12.5	10.9	7.9	10.4
2000	11.6	9.6	7.3	9.5
Irrigation Mean	10.9	8.9	6.4	8.8
LSD 0.05	Irrigation = 0.2 Humic acid = 0.2 Irrigation × Humic acid = 0.3			

Inflorescence diameter

The shorter the irrigation interval, the smaller the inflorescence. The mean of the irrigation-factors was 8.94, 8.20, and 7.26 cm for the 3, 6, and 9 days interval respectively. The 1000 mg/L treatment was supe-

rior with a mean of 8.89 cm with the most superior treatment-combination was the treatment combination of I_{1H1} (8.89 cm).

Table 4. Effect of irrigation intervals and humic acid on inflorescence diameter

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	8.10	7.12	6.11	7.11
1000	9.61	8.93	8.12	8.89
2000	9.11	8.55	7.56	8.41
Irrigation Mean	8.94	8.20	7.26	8.13
LSD 0.05	Irrigation = 0.09 Humic acid = 0.09 Irrigation × Humic acid = 0.15			

Plant pigments

The increase in the irrigation interval decreased the total chlorophyll by 2.86–1.78 mg/g fresh weight and carotenoids by 0.58–0.36 mg/g fresh weight. The 1000 mg/L treatment recorded the highest mean

value for both pigments and I_{1H1} had the highest value for each pigment. This is in line with the previously reported reactions of the geranium to drought-induced pigment (Mazrou *et al.*, 2023) [2].

Table 5. Effect of irrigation intervals and humic acid on pigment content

A. Total Chlorophyll				
Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	2.41	1.97	1.30	1.89
1000	3.21	2.80	2.14	2.72
2000	2.97	2.53	1.90	2.47
Irrigation Mean	2.86	2.43	1.78	2.36
LSD 0.05	Irrigation = 0.04 Humic acid = 0.04 Irrigation × Humic acid = 0.08			
B. Carotenoids				
Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	0.49	0.39	0.25	0.38
1000	0.66	0.57	0.42	0.55
2000	0.61	0.51	0.41	0.51
Irrigation Mean	0.58	0.49	0.36	0.48
LSD 0.05	Irrigation = 0.01 Humic acid = 0.01 Irrigation × Humic acid = 0.01			

Antioxidant enzyme activity

The activities of SOD, CAT, and POD increased as the irrigation interval was extended. The highest treatment-combination values occurred in I_{3H1} and were 2.38, 1.03, and 1.61 units/g fresh weight/min,

respectively. This pattern indicates defensive activation under severe stress, with a clearer stimulatory response at 1000 mg/L than at the higher concentration (Gerami *et al.*, 2025) [5].

Table 6. Effect of irrigation intervals and humic acid on antioxidant enzyme activity

A. Superoxide Dismutase SOD				
Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	

(continued on next page)

A. Superoxide Dismutase SOD

0	1.03	1.38	1.85	1.42
1000	1.28	1.79	2.38	1.82
2000	1.23	1.64	2.19	1.69
Irrigation Mean	1.18	1.60	2.14	1.64
LSD 0.05	Irrigation = 0.03 Humic acid = 0.03 Irrigation × Humic acid = 0.05			

B. Catalase CAT

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	0.42	0.57	0.72	0.57
1000	0.56	0.77	1.03	0.79
2000	0.51	0.70	0.92	0.71
Irrigation Mean	0.49	0.68	0.89	0.69
LSD 0.05	Irrigation = 0.02 Humic acid = 0.02 Irrigation × Humic acid = 0.04			

C. Peroxidase POD

Humic Acid (mg/L)	Irrigation Interval			Humic Acid Mean
	3 days	6 days	9 days	
0	0.60	0.86	1.21	0.89
1000	0.80	1.16	1.61	1.19
2000	0.72	1.09	1.44	1.08
Irrigation Mean	0.71	1.04	1.42	1.05
LSD 0.05	Irrigation = 0.02 Humic acid = 0.02 Irrigation × Humic acid = 0.04			

Discussion**Plant height**

A reduction in height with increased irrigation intervals was explained by the reduction in water potential and turgor pressure which decreased cell elongation and shoot-apex activity. The humic acid may be responsible for increased nutrient uptake, plant water status control and plant growth stimulation. Hammam *et al.* (2021) [3] reported increased plant height after the application of humic acid under various irrigation levels in geranium.

Shoot dry weight

Dry weight is a net result of photosynthesis and the development of leaves and branches, and will reduce if stomatal closure occurs again and carbon dioxide inflow is limited. This decrease is corroborated by the biomass reduction reported for *P. graveolens* with increasing water deficit and enhancement of water use efficiency to maintain productivity in the foliage (AbuEl-Leil *et al.*, 2024) [1].

Number of inflorescences

The inflorescence formation needs constant provision of carbohydrates, water and nutrients; stress may thus result in a shift of resources to survival instead of floral differentiation. The higher number of inflorescences at 1000 mg/L indicated that humic acid provided nutritional status and photosynthesis enough to sustain floral-bud formation, which was mentioned by Elhindi *et al.* (2023) [4] on ivy geranium treated with humic acid.

Inflorescence diameter

The diameter of inflorescence is affected by the expansion of the flower-cells and by the amount of assimilates available for the growth of the inflorescence. In water deficit situation, the decrease in expansion and size of floral tissue is limited by application of humic acid which also enhances nutrient use and physiological status. The relative advantage of 1000 mg/L was more consistent than 2000 mg/L, suggesting that there is an optimum dose beyond it which further increase would not be guaranteed (Elhindi *et al.*, 2023) [4].

Total chlorophyll

The lower level of chlorophyll was correlated with increased photooxidation, disruption of chloroplast membranes, and a decrease of pigment synthesis, which may be responsible for part of the increase

in dry weight and flowering that was observed with humic acid use, as it aids in maintaining pigment levels and thereby aids in the retention of the leaf's ability to capture light. In the case of geranium, a foliar treatment reduced the effects of drought, resulting in an increase in chlorophyll content and growth under water stress (Mazrou *et al.*, 2023) [2].

Carotenoids

Carotenoids play a role in light harvesting, dissipation of excess energy and protection of photosynthetic system against oxidation; their content may decrease if the capacity for biological renewal is insufficient to cope with oxidative damage. The results of the model indicate that the humic-acid-treated plants were better able to retain the higher values, possibly as a result of their improved chloroplast protection or tissue nutrition. The relationship between foliar biostimulant and drought is similar to the one reported for carotenoid response in *P. graveolens* (Gerami *et al.*, 2025) [5].

Superoxide dismutase SOD

SOD activity is the first line of defense as it transforms the superoxide radicals to hydrogen peroxide, and therefore the activity of SOD is usually elevated during increased oxidative pressure. The increased activity after every 9 days under irrigation is indicative of acclimatory response, and the increased activity at 1000 mg/L indicates better defensive priming. Under water deficiency conditions, the activity of SOD (superoxide dismutase) has been shown to increase in the leaves of geranium (AbuEl-Leil *et al.*, 2024) [1] and after foliar application, it has been shown to be modified (AbuEl-Leil *et al.*, 2024) [1].

Catalase CAT

When the model is modified by adding more CAT, the rate of hydrogen peroxide consumption increases in the model as it is quickly turned into water and oxygen. The response of the CAT, however, can go both ways depending on the severity of the stress, length of exposure, and condition of the tissue; either the activity may increase upon successful defense or decrease when the stress is severe and an inhibitor acts on the enzyme. The activity of CAT was controlled by melatonin treatment in geranium and found to be correlated to decreased oxidative damage under drought (Mazrou *et al.*, 2023) [2].

Peroxidase POD

POD is involved in hydrogen peroxide elimination through electron donors that are phenolics, and is also linked with cell-wall stiffening. Its rise with long irrigation times would thus be a reaction of defense and its superiority in 1000 mg/L suggests that humic acid also contributed to the enzymatic capacity along with growth. Under drought, foliar application of humic acid stimulated antioxidant mechanisms and enhanced the physiological efficiency in maize (Altaf *et al.*, 2023) [11].

Interaction between irrigation and humic acid

The interaction in the model was significant, suggesting that the amount of response of the plants to the humic acid was not consistent at each irrigation interval. The relative compensatory effect seemed to occur at 6 and 9 days, but the absolute compensation was overall not good under irrigation every 3 days. This finding indicates that this biostimulant should be tested on the same stress level and not just the overall mean, as the effectiveness of humic acid under different water regimes and cultivar has been confirmed in recent experiments (Al-Demrashed *et al.*, 2025) [12].

Conclusions

The experiment revealed that the irrigation interval can be prolonged up to 9 days, which may decrease the plant height, shoot dry matter content, number and diameter of inflorescence, as well as the amount of pigments, and activate the defense system of the plants which include the activity of the enzymes SOD, CAT, and POD. The other effects were mitigated by foliar humic acid, and 1000 mg/L was more effective than 2000 mg/L in most traits. Irrigation every 3 days with 1000 mg/L gave the maximum growth, flowering and pigment value. The highest enzyme activity was observed when watered every 9 days with 1000 mg/L, which was in response to defend against but not necessarily related to higher productivity (Elhindi *et al.*, 2023) [4].

Recommendations

To check if the time between irrigations is consistent stress levels under site conditions (Elsayed *et al.*, 2023) [6], repeat the experiment seasonally using the same treatment levels with the growing media wetness measured before each irrigation and the amount of water for each irrigation recorded.

Start with 1000 mg/L as a practical concentration of humic acid, add intermediate levels such as 500 mg/L and another level in between 1000 and 2000 mg/L to set up the response curve and optimal dose (Elhindi *et al.*, 2023) [4].

Add relative water content, stomatal conductance, proline, MDA, and H₂O₂ measurements to the list as these parameters help correlate reduced growth with the level of oxidative damage and the protection mechanism (Mazrou *et al.*, 2023) [2].

Repeat the experiment more than one season or environment and do an Economic analysis of water use and spray cost. Productivity as well as water-use efficiency should then be used as the final recommendation (Hammam *et al.*, 2021) [3].

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How to cite this article: Aboud KH. Effect of foliar humic acid application and extended irrigation intervals on vegetative, floral, and chemical traits and antioxidant enzyme activity in geranium. *International Journal of Agriculture and Food Science*. 2026;8(9):770-774. doi: <https://www.doi.org/10.33545/2664844X.2026.v8.i9k.1949>